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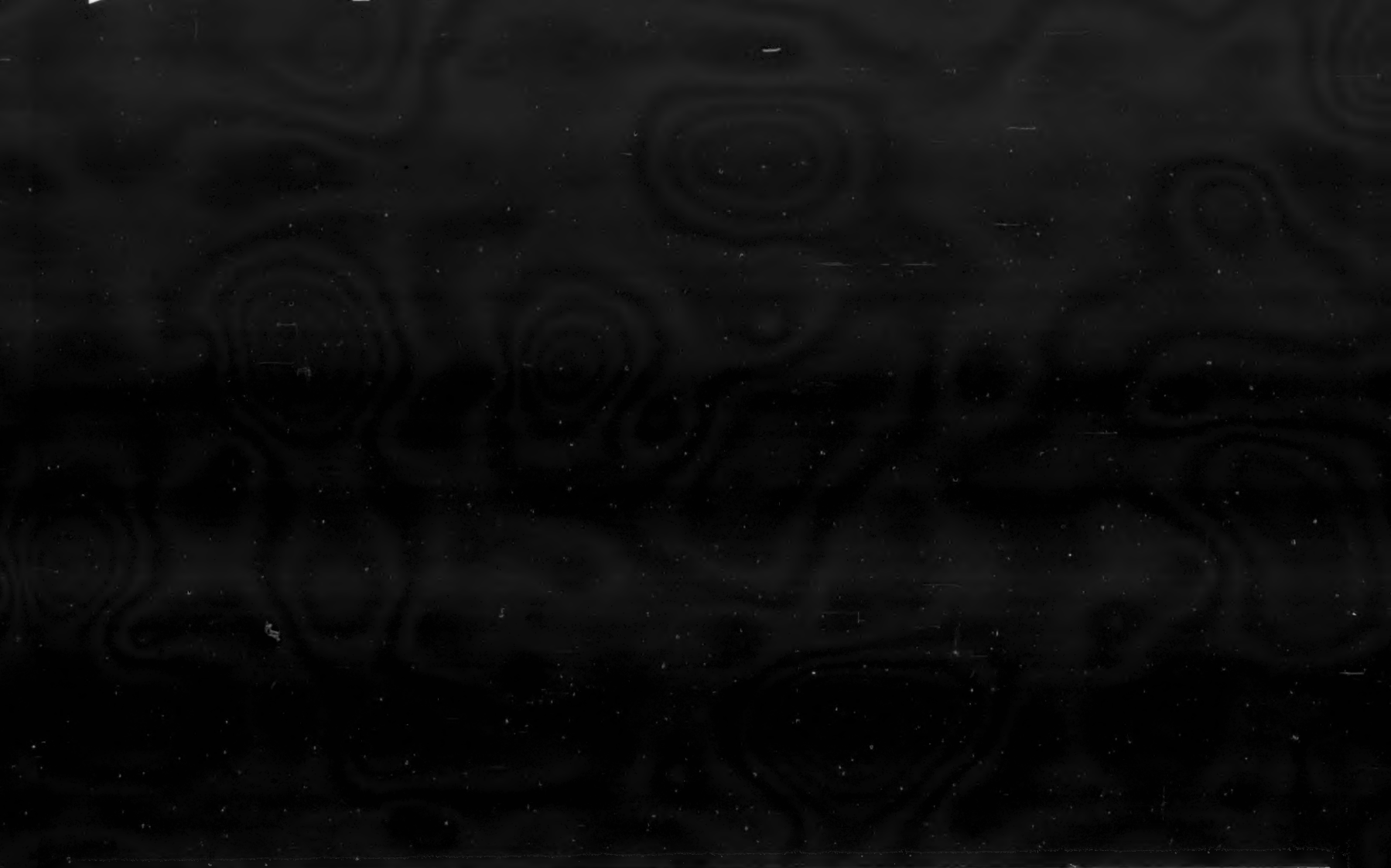

NOTICE

In the binding of this issue of the Canadian Journal of Botany, Plate I for the paper *Sclerotinia borealis in Canada* by J. Walton Groves and Constance A. Bowerman was placed in the paper *Ecological study of the peat bogs of Eastern North America. II. The Chamaedaphne calyculata community in Quebec and Ontario* by Fernando Segadas-Vianna, and vice versa. An unbound copy of each of these plates is therefore provided and should be inserted as follows:

Groves and Bowerman: *Sclerotinia borealis*,—opposite page 592.

Segadas-Vianna: Peat bogs. II,—opposite page 650.

Canadian Journal of Botany

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THE PRODUCTION OF MUTATIONS BY THE IRRADIATION OF MONTCALM BARLEY[1]

BY THOMAS LAWRENCE[2]

Abstract

Five radiation sources were used to induce mutations in barley. All treatments were given at a dosage of 10,000 r. equivalent. The radiation sources with their respective dose-rates in the region of the irradiated seeds were: a betatron (181.8 r./min.), an X-ray machine (201 r./min.), radium–beryllium (5.3 r./min.), and two Co^{60} sources (4.5 r./min. and 75.75 r./min.). None of the radiation sources used was more effective than the X-ray treatment in producing mutations. The betatron and the high dose-rate treatments from Co^{60} appear to be somewhat less effective than X-rays. Over 30 different mutant types were produced, including a number of vital mutants, such as stiff-strawed and early-maturing types. These appear promising as new varieties, but require further agronomic evaluation. It is concluded that mutation induction will become a useful new approach for plant breeders.

Introduction

Plant-breeding work in many self-pollinated crops has been so exhaustive in utilizing readily available plant material that further varietal improvements may, as far as conventional methods are concerned, be achieved in only two ways: first, by the addition of one or two valuable characteristics, such as disease resistance, to a suitable existing variety; or second, by bringing new genetic variation into hybrid combination by new interspecific crosses. In other words, breeding work in the more important cereal crops is running rather low in new germ plasm, the basic raw material.

This situation has created a need for finding new sources of variation. Since this is becoming increasingly difficult to do by conventional methods, the production of mutations by irradiation may provide much needed and readily available new variability for utilization in the further improvement of self-pollinated crops.

Research in this field appears to have been somewhat discouraged in North America by Stadler's (39) disparagement of induced mutations as a source of plant-breeding material. The present work was stimulated by a Swedish

[1] *Manuscript received June 27, 1955.*

Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alberta. Based on a thesis submitted to the School of Graduate Studies, University of Alberta, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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report (18) on X-ray-induced vital mutants in barley which have possibilities as new commercial varieties or as good parental materials.

The objectives of the present project were fourfold:

(*a*) To produce agronomically useful mutants of Montcalm barley.

(*b*) To confirm results previously obtained at the University of Saskatchewan.

(*c*) To obtain a comparison of the mutagenic value of various radiation sources.

(*d*) To make at least a preliminary evaluation of induced mutation as a new technique in plant breeding.

Literature Review

Muller (30), working with drosophila, and Stadler (40), working with barley, were the first to report on the biological effects of radiations. Both reported that mutations could be produced artificially by irradiation of biological materials. This opened up a new field for biologists, especially geneticists and plant breeders.

Swedish workers have reported irradiation-induced mutations of various physiological and morphological types (9, 10, 12, 13, 14, 17, 18). Nybom (35) in a recent publication gives a summary of the various mutations which Swedish workers have obtained from irradiation studies. These studies show that radiations induce a wide range of mutations, ranging from very slight, almost undetectable changes to drastic, easily-observed morphological changes, and from lethal, economically-useless mutations to valuable new mutations which can be used as new varieties.

Opinions expressed in the literature concerning the usefulness of radiation-induced mutations as a method or tool in plant breeding may be arbitrarily divided into two general categories, favorable and unfavorable.

Plant breeders not in favor of the use of induced mutations base their objections on the premise that characteristics of value in breeding are more likely to be found in a varietal collection since variability in that state is more likely to occur without the many undesirable gene mutations and chromosome aberrations frequently found in irradiated progenies. Those of a favorable mind assume the burden of proof, and base their opinions mainly on experimental evidence such as that presented in this paper.

Stadler (39) who first induced mutations in plants by irradiation did not believe that this technique offered much as a tool in plant breeding. His objections were essentially those outlined above. Stadler reported further that before induced mutations could be of use to plant breeding a method of selective induction would have to be found.

He does, however, suggest two possible uses for induced mutations in plant breeding. First, in inbred lines of corn, induced mutations may be used to produce variability so that selection can be carried on further. Secondly, and with some enthusiasm, he suggests the use of induced mutations in fruit-tree breeding. Most of these trees are highly heterozygous and vegetatively

reproduced for commercial purposes. Their long reproductive cycle makes breeding by hybridization and subsequent selection a long, tedious, and often unrewarding job. But by induced mutations a new variation may be directly created which, because it may be vegetatively propagated, avoids the complications related to sexual reproduction.

Gustafsson (10, 13) reports that the plant breeder cannot neglect artificially induced mutants in the further improvement of his varieties. He points out that mutants must be evaluated on their merits as new varieties or as parental material.

Gustafsson and Tedin (19) report that the process of mutation can to some extent be controlled and directed by artificial means. Such a control is also suggested by Nilan (32). Gustafsson and Tedin (19) believe that mutation research is indispensable for the progress of plant breeding.

Oltman (36) predicted that by X-irradiation of plants it would be possible to obtain characters and qualities which have not yet been discovered in naturally variable material. Moreover, he reports that by induced mutations variations may be produced in crop material which has been thoroughly investigated by breeding and in which only small improvements can be produced by ordinary methods of breeding.

Several workers have obtained disease-resistant mutants by irradiation of plants. Ulonska (42) and Hoffman (22) report the induction of a mildew-resistant form of spring barley, otherwise identical with the original variety. Similarly, Bandlow (2) reports finding a mildew-resistant form of winter barley, apparently the first time that such resistance has been reported in this crop. Tolbert and Pearson (41) reported that X-ray treatment of the groundnut has resulted in mutants with an increased range of variability in resistance to leaf spot. Konzak (24) reported that rust-resistant mutants were obtained in oats after exposure to radioactive materials. He believes that mutants of agronomic value are to be found in irradiated populations. MacKey (28) has obtained a series of rust-resistant mutants in wheat.

Stiff-strawed mutants in barley have been reported by several workers (9, 13, 18, 22, 33, 35, 37). They have also been obtained in wheat (28). Gustafsson (13) reports obtaining three stiff-strawed mutants in barley at least equal in yield to the parent varieties. Similar results were obtained at Saskatoon (37).

Reports of other induced mutants of agronomic value are numerous. Increased earliness (13, 18, 22, 29, 33), increased drought resistance (16, 22), superior baking quality (22), and a wider range in protein content (range of parent 10–11%, range of mutant 7–16%) have been reported (22). Swedish workers have distributed two X-ray-induced varieties, one in white mustard, "Primex", which produces 7% more oil per hectare than the mother strain, and one in peas, "Stralart", which has a seed yield of 5% more than the original variety (1, 6).

Several workers agree with Stadler on the possibilitities of induced mutations in trees and shrubs. Granhall *et al.* (7) and Granhall (8) report that results

from X-rayed apple trees are very encouraging from a plant breeding point of view. Bishop (3) suggests that X-rays may be used as an artificial method of inducing color-gene mutations in apples. Lewis (27) suggests the use of X-rays to produce dwarf plants, which may be used as precocious root stocks in fruit-tree breeding, especially in the case of cherries and pears where the need has not been met by natural material. He also suggests that X-rays may be used to produce self-compatible plants in species which are normally self-incompatible. Mutations have been reported in other cross-pollinated crops. Hagberg and Nybom (20) report two instances of X-ray-induced mutations in potatoes. Julen (23) reporting on X-ray treatment of *Poa pratensis* obtained one mutant that seems to be better than the normal plants.

As late as 1950 we have an adverse opinion from Hagedoorn (21), a Dutch geneticist, who appears very dubious about the use of induced mutations in plant breeding.

On the whole there appears to be a great deal of controversy over the degree to which induced mutations may prove useful in a plant-breeding program. However, the favorable experimental evidence appears to be very substantial.

Much of the irradiation work conducted on barley has been based on treatments of the seeds.

A number of workers (4, 11, 26, 31, 32, 34, 38) report on various modifying factors and characteristics of the plants which alter the reaction of the treated seeds to irradiation. Six-rowed varieties are reported to be more sensitive to X-rays than two-rowed varieties (11). Larger seeds show greater germinability after X-ray treatment than do smaller seeds. Also the hull of barley and oats make hulled varieties less susceptible to radiation damage than hull-less varieties (4). Several reports indicate that susceptibility to X-ray damage decreases with an increase in polyploidy (26, 31, 38). A recent Swedish report (28) suggests that a higher observable mutation frequency may be obtained by irradiation of a polyploid than by irradiation of a diploid because of the better tolerance by the polyploid of chromosomal disturbances. Gelin (5) reported on the effects of increased moisture content on susceptibility of the seeds to X-ray damage. He found that an increase in mutation rate was achieved by increasing the moisture content of the seeds to be irradiated.

Materials and Methods

Montcalm, the barley variety used in this study, is a blue-seeded, six-rowed, smooth-awned type with a moderately strong straw and high malting quality. It is susceptible to loose smut and leaf rusts, but semiresistant to covered smut. It is one of the most widely grown varieties of malting barley in Western Canada.

The radiation sources with their respective dose-rates in the region of the irradiated seeds were: an X-ray machine operated at 200 KVP and 20.5 ma. with no filter and a dose-rate of 201 r. per minute; a betatron operating at

24 Mev. with a dose-rate of 181.8 r. per minute; a radium–beryllium source with a dose-rate of 5.3 r. per minute; and two Co^{60} sources, one with a dose-rate of 75.75 r. per minute (Co^{60} high), the other with a dose-rate of 4.5 r. per minute (Co^{60} low). All treatments were made at 10,000 r.

A special mechanical device was developed to adapt the radium–beryllium source to treatment of seeds. The device consists of a central plastic cylinder, containing the source, surrounded radially by a number of small plastic cylinders, in which the seeds are placed for treatment. Each of the seed containers revolves individually, and they all revolve as a group around the central cylinder containing the source. The device enables the treatment to be done with very little trouble. The operator has only to put the seed in the seed cylinders, put the source in the central cylinder, turn the device on, and leave it running for the desired length of time. The time will depend on the dosage desired.

The seed was treated and the X_1 grown at the University of Saskatchewan in the summer of 1952. In the fall of 1952 the material was harvested and taken to the University of Alberta. The number of X_1 plants harvested for further study and the treatments used are given in Table I.

In 1953 a maximum of five tiller-progenies from each X_1 plant were grown at Edmonton. The resulting X_2 population consisted of approximately 10,000 tiller-progenies. A check (Montcalm) was grown in every 10th row throughout the entire nursery.

In 1954, an X_3 generation, comprising most of the mutants found in the X_2, was grown at Swift Current, Sask. This population consisted of approximately 5000 progeny rows.

In the present investigation, as in most plant-breeding projects on induced mutations, the population handled was too large to permit the use of special techniques for the detection of less obvious mutations. The range in mutations reported herein is thus limited to readily observable types.

TABLE I

DETAILS OF TREATMENTS

X_1 plants, No.	Treatment	r./min.	Length of treatment, in min.
500	10,000 r. $Co.^{60}$ high	75.75	133.20
500	10,000 r. Co^{60} low	4.5	2221.80
500	10,000 r. X-ray	201.0	49.75
500	10,000 r. betatron	181.8	55.00
300	10,000 r. radium–beryllium	5.3	1875.00
500	Checks		

Experimental Results

Observed Effects of Treatments in X_1

The irradiation treatments were made on lots of 1000 seeds each during the period April 3–7, 1952. This seed was stored until May 2, at which time it was sown. Seedling emergence was very poor. Consequently additional seeds were treated during the period June 2–6 and were sown in the field directly after treatment. The emergence from the latter treatments was good. Data on number of seeds treated and their emergence are given in Table II. Only one lot of seeds was treated with radium–beryllium owing to the unavailability of the source at the time of the second treatment. The results indicate that storage of seeds after treatment may reduce their ability to survive irradiation treatments. Similar findings are also reported by Gustafsson (13) and Stadler (39).

Several twin heads were observed in the X_1. Seed from these plants gave rise to normal-appearing progeny.

Some sterility was observed in the late-sown X_1 material but, since it could not be determined with certainty whether this effect was due to the treatment or due to frost damage, no attempt was made to estimate the degree of sterility.

Observed Effects of Treatments in X_2

The X_2 in irradiation work may be compared with the F_2 of an ordinary cross, for in both cases segregation occurs. In the X_2 the mutants which are very preponderantly recessive segregate out and become visible, much as in F_2 segregation for recessive phenotypes.

TABLE II

Data on number of seeds subjected to each treatment and on number of seedlings subsequently emerged

Lot No.	Treatment	Seeds treated, No.	Seedlings emerged, No.	Date treated
1	Co^{60} (high)	1000	57	April 5
2	Co^{60} (high)	2612	1098	June 2
1	Co^{60} (low)	1000	55	April 5–7
2	Co^{60} (low)	3035	1496	June 3–6
1	X-ray	1000	95	April 5
2	X-ray	2850	1442	June 4
1	Betatron	1000	103	April 3
2	Betatron	2424	1383	June 5
1	Radium–beryllium	1000	343	May 6–14

For purposes of description the mutants found in the X_2 were divided into three general classes as follows:

1. Chlorophyll mutants.
2. Stiff-strawed mutants.
3. Other mutants.

A description of the mutants found, with notes on their behavior in the X_3 (when that generation was grown), follows:

1. Chlorophyll Mutants

The most readily detectable mutants observed in the X_2 were the chlorophyll-deficiency types, most of which were lethal, dying when the food supply was exhausted in the endosperm. Invariably these mutants appeared from only one tiller–progeny of an X_1 plant.

(*a*) *Albinos*

The albinos were pure white seedlings, and were the most frequently occurring of all the mutants observed in the project. The albino character was lethal. The progeny of plants heterozygous for this character segregated in a ratio of three green to one white.

(*b*) *Yellows*

These mutants were similar to the albinos in that the character was lethal and in that the progeny from heterozygous plants segregated in a 3 : 1 ratio. They had a color varying from light yellow to a golden yellow and were quite frequent in occurrence.

(*c*) *Orange*

The orange mutants were similar to the yellows except for their color, which was an orange or reddish yellow shade. They were rare in occurrence, lethal, and the progeny from heterozygous plants segregated in a 3 : 1 ratio of green to orange seedlings. This type of mutant was only noted in the X-ray-treated material.

(*d*) *Yellow-green*

These plants, about equal to the yellow mutants in occurrence, were somewhat more yellowish than the normal seedlings, and gradually became greener until they were indistinguishable from the normal at the mature-plant stage. The progeny of heterozygous plants segregated in a ratio of three green to one yellow-green and homozygous plants breed true, as indicated by X_3 results.

(*e*) *White, Green-tipped*

These mutants are very similar to the albinos except that the tips of the leaves are green. They were of comparatively rare occurrence, and died soon after emergence. The progeny of heterozygous plants segregated in a 3 : 1 ratio.

(*f*) *Yellow, Green-tipped*

These plants are identical with type (*e*) described above, except that they were yellow where type (*e*) were white. They were of rare occurrence, none

being found in the progeny from the seeds treated with X-rays. The progeny of plants heterozygous for this mutant segregated in a 3 : 1 ratio. In most cases this character was lethal in the seedling stage; however, one or two plants survived to the mature-plant stage but failed to set seed.

(*g*) *Variegated or Striped*

Variegated plants, for the most part white plants with green stripes, were found in all treatments except Co^{60} low. These plants usually survived until maturity but because they had a slower growth rate they were late and were frozen before ripening. The progeny from heterozygous plants segregated in a 3 : 1 ratio of green to variegated.

In addition to the variegated plants described above green plants with yellow striping were found in the betatron and Co^{60} high treatments.

(*h*) *Mottled*

Several plants were observed in the radium–beryllium treatment that were green with yellow spots, giving a mottled effect. The yellowish spots gradually became greener until at the mature-plant stage this mutant was indistinguishable from the normal. Plants heterozygous for the mottled character segregated in a 3 : 1 ratio.

(*i*) *Grandpa*

These plants were found in the radium–beryllium and Co^{60} low treatments. Their leaves had considerable to very little variegation with white. The leaf sheath and culm were invariably white. The heads, awns, and aleurone were all white. This mutant was similar to one described by Martini and Harlan (29).

2. Stiff-strawed Mutants

Several plant progenies observed in the treatments were found to have considerably stronger straw than the adjacent Montcalm checks. Two distinct types of mutants which can be classified as stiff-strawed were observed.

(*a*) *Stiff-strawed*

This mutant was designated as stiff-strawed, for want of a more suitable name. These plants seemed to be stronger throughout the entire culm. They were distinguished by the erectness of the heads, which was in sharp contrast to the nodding heads of the Montcalm plants. They seem to have an especially strong neck. This type of mutation was found in all treatments but Co^{60} high, and bred true in the X_3. These mutants may turn out to be the most useful mutant as far as increased straw strength is concerned. A similar mutant obtained at Saskatoon (37) has shown up well in yield tests and appears to be equal to Montcalm in yield and superior to it in straw strength.

(*b*) *Dense-headed*

Mutants similar to this type were designated as erectoides by Swedish workers.

Although all plants in this group had dense heads, the density varied somewhat from one line to another. The heads were usually shorter than those of Montcalm and were held very erect, and the kernels were slightly smaller. A strong straw was indicated by this erectness of the heads and also by their good resistance to lodging. In all cases there were a number of observable characteristics associated with the dense head, suggesting that this character may be pleiotropic. This is supported by a report of Gustafsson (13) in which he states that erectoides are pleiotropic.

The progeny of plants heterozygous for the mutant segregated in a ratio of 3 normal to 1 dense, and homozygous plants bred true as indicated by X_3 results. Dense-headed mutants were found in all treatments but Co^{60} low. The dense, compact type of head is shown in Fig. 1, Nos. *2*, *3*, and *4*.

(*c*) *Weak-strawed*

These mutants showed a definite weakness of the stem and were lodged consistently while the adjacent Montcalm checks were quite erect. This type was noted only in the betatron treatment.

3. Other Mutants

(*a*) *Early Maturing Mutants*

The mutants for earliness headed and matured from a week to 10 days earlier than the checks. They were found in all five treatments, with the majority being observed in the Co^{60} low and the radium–beryllium treatments. These mutants are of much practical interest because of the need for early varieties in northern agricultural regions.

(*b*) *Hooded Mutants*

Three hooded plants were observed in one X_2 tiller progeny row of an X_1 plant from the X-ray treatment. (See Fig. 1, No. *5*, and Fig. 7.)

The hood differed considerably from that of Warrior (a standard hooded variety). It was considerably broader than that of Warrior and terminated in a short curly awn. In most cases there was an opening in the top of the floret and the florets were sterile. The few seeds obtained in the X_2 bred true for the hooded character in X_3. The progeny of plants heterozygous for the character segregated in a 3 : 1 ratio of normal to hooded.

(*c*) *Singed*

A whole X_2 tiller progeny row of an X_1 plant from the betatron treatment showed a mutant character designated as singed. (See Fig. 1, No. *6*.) The mutant appeared normal except for the awns, which extended only a short distance beyond the top of the head, if at all, and their tips were curled. The singed character was also noted in the X_3.

(*d*) *Leafless*

This mutant appeared in the X_2 from seed treated with X-rays. It was characterized by a drastic reduction in size of leaves or, in some cases, a

complete absence of leaves. (See Fig. 9.) This character was also observed in the X_3 but the reduction in leaf area was not as drastic, suggesting that the environment may influence its expression.

(*e*) *Spindly or Vine-like*

These plants occurred in the X_2 of seeds treated with X-rays. The plants were long, thin, vine-like, prostrate, and light green in color (see Fig. 5). The heads were lax, poorly developed, and sterile. Plants heterozygous for this mutant segregated in a ratio of 3 normal to 1 spindly.

(*f*) *Tweaky*

One X_2 tiller progeny row from seeds treated with the Co^{60} low source showed tweaky plants. These plants have heads with several florets missing at one or more locations on the rachis, thus leaving a bare space. The tweakiness was not very strongly expressed in the X_3, indicating that its expression may be influenced by environment.

(*g*) *Short-awned*

One mutant appeared, in the X_2 of the Co^{60} high treatment, which had very fine, smooth awns. The awns of this mutant were also much shorter than the normal, extending about one-half as far beyond the head as do those of Montcalm. These plants bred true for short awns in the X_3.

(*h*) *Bushy-awned*

These plants were observed in the X-ray, Co^{60} low, and Co^{60} high treatments. The heads were almost completely sterile, the florets being composed of a series of hulls progressively surrounding each other to the main outer hull of the floret. The heads thus had the appearance of being fertile but only very few seeds were found. Each floret had 3 to 10 awns extending from it, thus giving the plants a bushy appearance (see Fig. 2, Nos. *7*, *8*, *9*, and *10*).

(*i*) *Waxy*

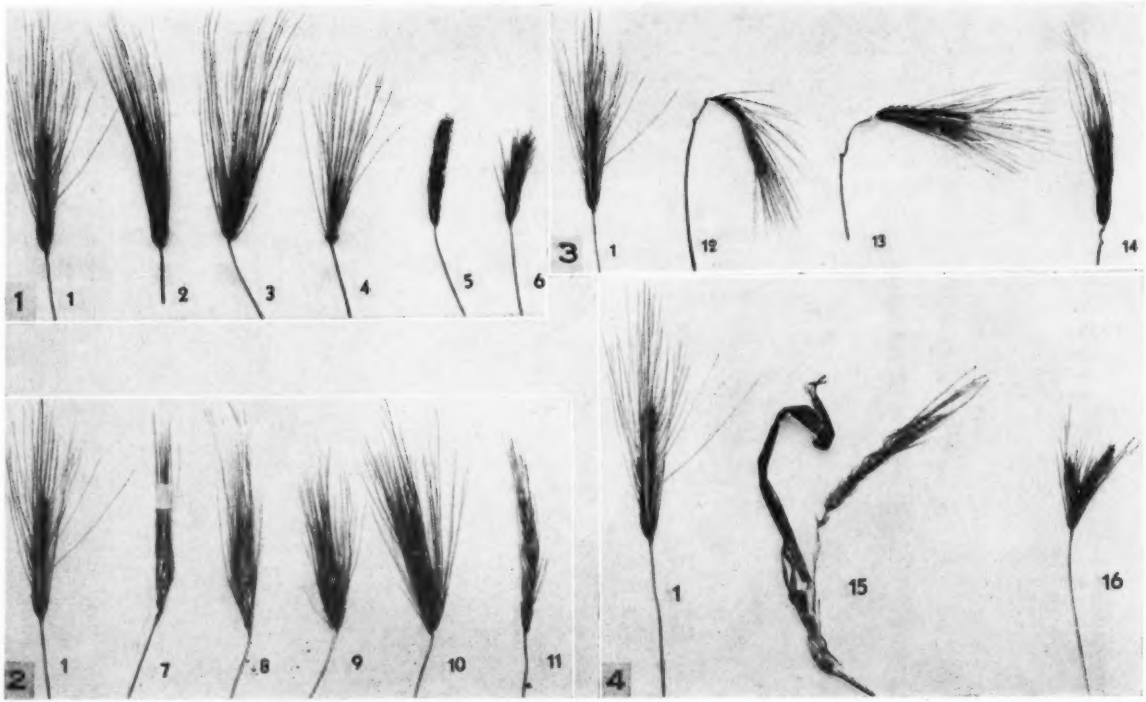
Several plants in one X_2 tiller progeny row from the betatron treatment had a very shiny, waxy appearance. These plants bred true for the waxy appearance in the X_3.

(*j*) *Multiflorous*

A multiflorous plant was observed in the betatron-treated material. It had supernumerary florets giving a somewhat bushy appearance to the heads. This mutant bred true in the X_3.

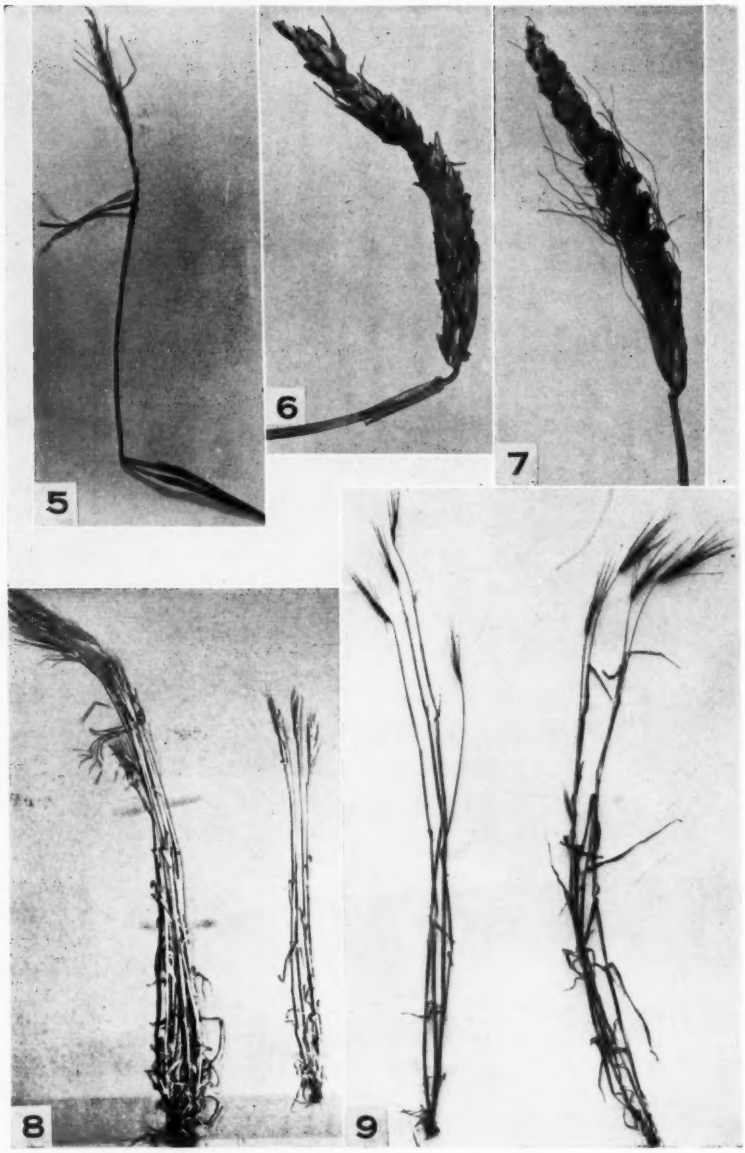
(*k*) *White Heads*

This mutant appeared to be identical with Montcalm until heading out, at which time it was noted that the heads were white. This whiteness was evident until the ripened heads became weathered. This mutant differed from grandpa in that only its heads were white and in that it was the same height as Montcalm. Grandpa, on the other hand, invariably had a white culm, usually variegated leaves, and was somewhat shorter than the Montcalm checks. The white head mutant was not noted in the X_3.



FIGS. 1, 2, 3, and 4. Photographs of barley mutants.

FIG. 1. *1*, Montcalm; *2*, *3*, *4*, dense-headed; *5*, hooded; *6*, singed. FIG. 2. *1*, Montcalm; *7*, *8*, *9*, *10*, bushy-awned; *11*, curly laterals. FIG. 3. *1*, Montcalm; *12*, *13*, *14*, elongated rachis internode. FIG. 4. *1*, Montcalm; *15*, elongated rachis internode; *16*, branched head.



FIGS. 5, 6, 7, 8, and 9. Photographs of barley mutants.

FIG. 5. Spindly or vine-like. FIG. 6. Absent lower laterals. FIG. 7. Hooded. FIG. 8. Left, Montcalm; right, dwarf. FIG. 9. Left, leafless; right, Montcalm.

(*l*) *Absent Lower Laterals*

This mutant occurred in the Co^{60} low treatment. It was characterized by a lack of lateral florets on the basal part of the head. The plants bred true for this character in the X_3. (See Fig. 6.)

(*m*) *Curly Laterals*

Another mutant occurred in this treatment (Co^{60} low) on which the awns of the lateral florets were curly or wavy. This bred true in the X_3. (See Fig. 2, No. *11*.)

(*n*) *Elongated Rachis Internodes*

In the X-ray, Co^{60} low, and Co^{60} high treatments mutants were observed which had elongated rachis internodes. (See Fig. 3, Nos. *12*, *13*, *14*, and Fig. 4, No. *15*.) This character bred true in the X_3. The type shown in Fig. 4, No. *15*, produced plants in the X_3 which were very broad leaved, late, and which in most cases failed to head. Those plants which did head out failed to set seed before being killed by fall frosts.

(*o*) *Narrow-leaved*

The radium–beryllium treatment gave rise to a mutant having slim, narrow leaves. Unfortunately this mutant could not be observed in the X_3 owing to accidental damage.

(*p*) *Branched Head*

One plant with a branched head was observed in the X_2 of the X-ray-treated material. This plant produced only normal plants in the X_3. (See Fig. 4, No. *16*.)

(*q*) *Dwarf Plants*

In the summer of 1953 numerous dwarf plants were noted in all treatments and also in the check materials. (See Fig. 8.) These dwarf plants were also observed in plots of other barley varieties such as Gateway. A number of the X_2 dwarf plants from the treated material bred true for the dwarf characteristics, some from the treated material and all from the checks appeared normal in the X_3. The number of dwarf plants observed in the nursery in the X_2 was so great that they could not all be checked in the X_3, so differences in the number of dwarf plants from the different treatments could not be ascertained.

In addition to the numerous mutants listed above, a considerable number of sterile plants were found throughout the entire nursery.

Observed Effects of Treatments in X_3

Work on this generation was confined, first, to studies of segregation among X_3 plants derived from selected X_2 head rows that had shown mutations and, secondly, to increasing the seed of mutations of possible agricultural value with a view to further testing.

The results on mode of inheritance for the mutants tested are given in Table III.

TABLE III

SEGREGATION IN THE X_2 GENERATION, WITH χ^2 TESTS FOR GOODNESS-OF-FIT TO THE 3 : 1 RATIO

Type of mutant	Normal plants, No.	Mutant plants, No.	χ^2	P
Albinos	690	225	0.08	0.95
Yellows	812	257	0.62	0.50
Orange	500	184	1.32	0.30
Yellow-green	2526	832	0.09	0.95
White, green-tipped	284	85	0.77	0.30
Yellow, green-tipped	709	264	2.36	0.10
Variegated	419	134	0.18	0.95
Mottled	159	54	0.01	0.95
Dense-headed	551	174	0.39	0.50
Hooded	52	15	0.25	0.50
Spindly	91	35	0.56	0.50
Bushy-awned	123	32	1.57	0.20

MUTATION RATES RELATIVE TO SOURCE OF RADIATION

A summary of the distribution per treatment of the plant progenies which showed chlorophyll aberrants or vital mutations is presented in Table IV.

Gustafsson and MacKey (18) reported that after irradiation with 10,000 r. units of X-ray about 500, or about five per cent, chlorophyll aberrants could be expected from the progeny of 10,000 X_1 spikes. The aggregate results for all five treatments used in this study were in fairly close agreement with this expectation, but the results from the betatron (4.3%) and from the Co^{60} high (3.7%) treatments were somewhat lower.

TABLE IV

RATES AT WHICH CHLOROPHYLL-DEFICIENT AND VITAL MUTANTS WERE PRODUCED IN RELATION TO SOURCE OF RADIATION

Source	Level of treatment	Plant progenies grown, No.	Progenies with chlorophyll deficiencies, No.	Progenies with vital mutants, No.	Chlorophyll deficiencies, %	Vital mutants, %
Betatron	10,000 r.	1708	73	13	4.3	0.8
X-ray	10,000 r.	1765	103	19	5.8	1.1
Radium–beryllium	10,000 r.	1355	76	14	5.6	1.0
Co^{60} low	10,000 r.	1849	99	22	5.3	1.2
Co^{60} high	10,000 r.	1420	53	6	3.7	0.4
Checks	—	1581	3	0	0.2	—

The results for the betatron treatment in this experiment (4.3%) are strikingly different from those obtained in an earlier study (15.2%) (25). This result is probably due to the method of treatment. In the earlier study a phantom made of pressed wood was inserted between the seed being irradiated and the source. This phantom increased the density of ionization at the level of the kernels. In the present study no phantom was used.

Results from the two Co^{60} treatments indicate that the intensity of treatment has an effect on the mutation rate. The Co^{60} low treatment was made with a source having a dose-rate of 4.5 r. per minute at the level of the seeds and gave 5.3% chlorophyll mutants and 1.2% vital mutants. The Co^{60} high treatment was done with a source having a dose-rate of 75.75 r. per minute at the level of the seeds and gave 3.7% chlorophyll mutants and 0.4% vital mutants. This suggests a need for a more extensive study of this phase.

Discussion

The first objective of this project was to produce useful mutants in Montcalm barley. Stiff-strawed and early mutants, which may be of considerable agricultural value, have been obtained, but final assessment of their value will have to await further tests for yield and quality. Only these final tests can give the answer as to whether or not this objective has been successfully achieved. In any event, mutations have been obtained that are superior to the mother line in certain aspects and, although they may not be directly useful as new varieties, they may well serve as parental material in a hybridization program.

The second objective was to confirm results previously obtained at the University of Saskatchewan. This has been generally achieved, especially through the obtaining of stiff-strawed and early mutants. These results show that the induction of certain types of mutations by irradiation of plant material is reproducible indicating that the radiation technique can be used on any suitable variety or strain with good possibilities of obtaining economically important characters such as stiff straw and earliness.

The third objective was to obtain a comparison of the mutagenic value of various radiation sources. As shown in Table IV, none of the other radiation sources used was better than the X-ray machine relative to the number of mutations induced. However, the betatron and Co^{60} high treatments appear to be somewhat less effective than X-rays in this respect. These results may be explained by differences in the density of ionization. If phantoms had been used when making the betatron and Co^{60} high treatments the results might have been quite different. The X-ray treatment appeared to be most effective in producing the erectoid type of mutation. Three of these mutants were found in the X-ray treatment, none in the Co^{60} low, and one in each of the other three treatments. The X-ray treatment also gave a slightly greater range in mutant types than was found in the materials of the other treatments.

The fourth objective was to attempt an evaluation of the induced-mutation technique as a method of plant breeding. In considering this question and in

making a comparison between mutation induction techniques and other plant-breeding methods, one must consider the time and costs involved and the results achieved.

When one considers induced mutation in relation to the three conventional methods of plant breeding: introduction, selection, and hybridization, it does perhaps at first appear as if it were of doubtful value. But when one considers the limitations of these methods, the possibilities of the induced-mutation technique appears more promising. Introduction as a method of plant breeding is limited by the degree to which available exotic types may be adapted to a plant breeder's needs. Selection as a method of plant breeding is limited by the observational powers of the breeder and by the amount of variability of the crop. Hybridization as a method is limited by the quality of available parents and by the number of crosses that can be handled. The induced-mutation technique would also be limited by the amount of material that could be handled, but would not be limited by the amount of variability in the mother lines, nor by serious questions of adaptation to local conditions. Mutation induction might be used to produce new variability which would project the scope of selection and hybridization work well beyond the present limits of these methods.

The time element is a very important factor in plant breeding, and in this connection the induced-mutation technique may have a marked advantage over hybridization. When a recessive mutant of direct agronomic value appears in the X_2, it has the purity of the mother line and may be increased without further selection. In a hybridization program many generations of hybrids and often much selection work are necessary to attain comparable purity.

In the relatively short period of 27 years, induced mutation is rapidly becoming recognized as a new technique for plant breeding. Two years after the initiation of the present project 16 mutant lines are ready to be entered into yield tests. On the basis of these results it is concluded that induced mutations should have a place in a plant-breeding program and should no longer be neglected for this purpose.

Acknowledgments

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FOOT ROT OF WALLFLOWER CAUSED BY *PHYTOPHTHORA MEGASPERMA*[1]

By Walter Jones[2]

Abstract

A previously unreported foot rot disease of wallflower, *Cheiranthus cheiri*, caused by the fungus *Phytophthora megasperma*, was found in Saanichton, B.C., in 1953. The optimum temperature for growth of the fungus is 20° to 25° C., the maximum approximately 31° C., and the minimum below 5° C. Sporangia develop at all temperatures between 5° and 20° C., but the optimum temperature for their development is approximately 14° C., whereas the optimum temperature for the development of organs of fusion is approximately 20° C. The fungus proved to be pathogenic to wallflower plants only under wet soil conditions, hence it is considered that good soil drainage is an important factor in the control of the disease.

In March 1953, numerous overwintered plants of wallflower, *Cheiranthus cheiri* L., were found to be diseased or dead in two flower beds in Saanichton, B.C. (Fig. 1). The texture of the soil in the beds was heavy, and natural drainage was poor. During the previous winter, the weather was wet and mild, and the precipitation during January was the heaviest recorded for the district. The leaves of the diseased plants were pale green to whitish in color, and many of them had fallen. The basal parts of the stems of these plants were rotted, and there was considerable sloughing of the cortex. Some of the roots were also necrotic.

When diseased tissues were examined, many oospores of a species of *Phytophthora* were found in them, and after these tissues had been placed in water for approximately 24 hr. at 14° C., many sporangia developed from them (Fig. 3). Diameter measurements of the oospores were taken, and these were grossly similar to those of *Phytophthora megasperma* Dresch.

In an adjacent field, a stem rot of overwintered cabbage plants was also present, and the diseased tissues of these plants contained oospores with diameter dimensions similar to those of the oospores found in diseased wallflower tissue.

A foot rot of wallflower caused by a species of *Phytophthora* has not been previously reported, although Tompkins *et al.* (3) were able to infect wallflower plants with *P. megasperma* isolated from diseased cauliflower plants in California.

It seemed desirable therefore to determine the growth–temperature relationship and the pathogenicity of the fungus isolated from the diseased wallflower plants.

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The Fungus

The isolate grew readily on potato-dextrose agar and, after a few days, numerous organs of fusion with predominantly paragynous antheridia developed in culture (Figs. 4, 5, 6). The diameter dimensions of the oospores were 27.3 μ to 50.2 μ, an average of 40 μ, whereas those of *P. megasperma* as given by Dreschler (1) are 26 μ to 52 μ, an average of 41.4 μ. It is considered therefore that the morphological characters of the fungus are the same as those of *P. megasperma*.

The fungus was grown at different temperatures on potato-dextrose agar. The optimum temperature for growth was found to be 20° to 25° C., whereas the maximum was approximately 31° C., and the minimum below 5° C.

To determine the influence of different temperatures on the development of sporangia and organs of fusion, diseased tissues from artificially infected plants were placed in distilled water in Syracuse dishes, and then placed in incubators held at 5°, 9°, 13°, 17°, 21°, 25°, and 29° C. Within five days, sporangia had developed at all temperatures between 5° and 20° C. The optimum temperature for their development was approximately 14° C. Organs of fusion were found after six days in tissues incubated at 17° to 25° C., but the most favorable temperature for their development was approximately 20° C.

Pathogenicity

To determine the pathogenicity of the fungus to wallflower plants, a pure culture inoculum grown in vermiculite and corn meal was placed at the base of two young wallflower plants in the greenhouse. To encourage the development of sporangia, the inoculum was kept continuously moist by periodic drips of distilled water from a container. Two other plants were set aside for control. All the plants were one month old.

After a period of one month, symptoms of infection were not observed. Because of this negative result, one of the pots in the inoculated series and one in the control series were partly immersed in water until the soil was more or less waterlogged. After approximately two weeks under these conditions, symptoms of infection were evident in the inoculated plant, but the control plant remained healthy.

In another series, four young wallflower plants, which were three weeks old, were inoculated in a similar manner in the greenhouse. After two weeks, symptoms of infection were not evident, but when the pots were immersed in water, symptoms of infection became evident after two weeks. Four other plants which were not inoculated, but otherwise treated similarly, remained healthy.

The symptoms of the disease, following artificial inoculation of the plants, appeared as a 'flabby' wilting of the foliage (Fig. 2). The leaves became pale green, and later somewhat whitish in color, and the plants eventually died. In the affected plants, the stems below soil level and some of the fibrous roots

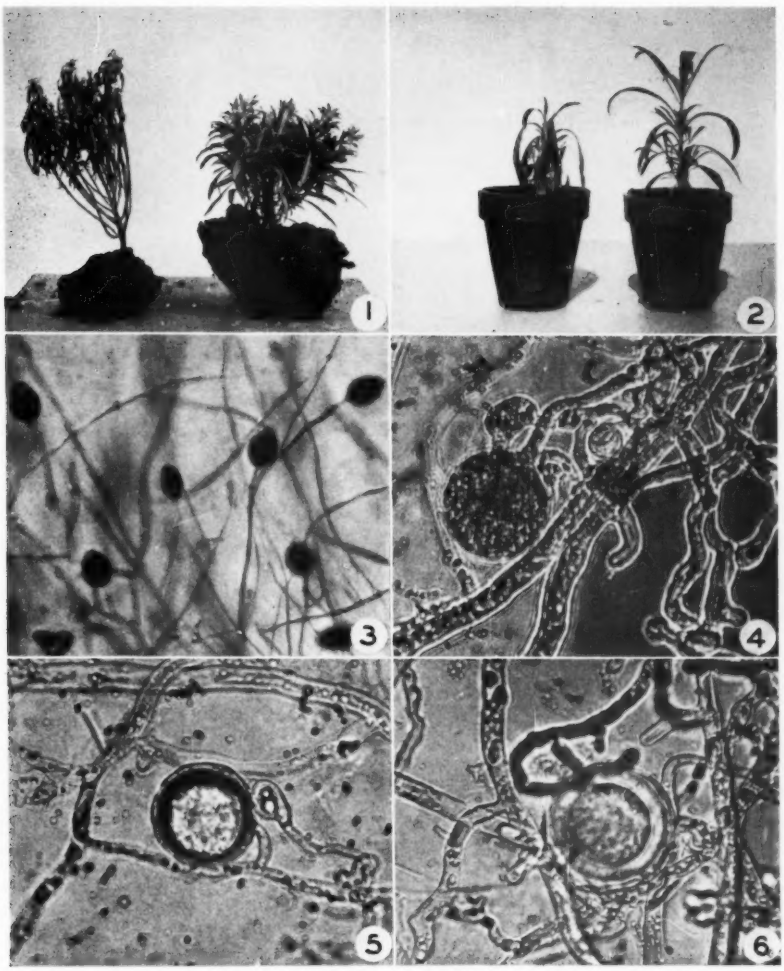

FIG. 1. Diseased and normal wallflower plants—field symptoms.

FIG. 2. Left—symptoms of the disease on artificially inoculated wallflower plant; right—control.

FIG. 3. Sporangia from diseased wallflower tissue.

FIGS. 4–6. Organs of fusion—stages of development showing: oogonium, paragynous antheridium, and oospores.

were partly rotted, and the steles of the necrotic fibrous roots were slightly discolored. When the tissues of diseased stems and roots were examined microscopically, numerous organs of fusion were found in them.

The results indicate that the fungus is pathogenic to wallflower plants only under ext mely wet soil conditions.

Discussion of Results and Control Measures

Results of the growth–temperature studies show that the fungus can grow and the sporangia can develop at relatively low temperatures. In the pathogenicity experiment, the results indicate that the fungus is pathogenic only under extremely wet soil conditions. These climatic and soil conditions are usually prevalent in the coastal areas of British Columbia during the late autumn and late winter. The results of pathogenicity experiments by other workers also indicate that *P. megasperma* can infect plants only under wet soil conditions. Thus Tompkins *et al.* (3) inoculated potted wallflower plants with *P. megasperma* isolated from cauliflower, and after the pots were placed in buckets of water, the plants gave evidence of infection. In their pathogenicity experiments, Robertson and Ogilvie (2) obtained positive proof of infection of young cauliflower plants after standing the pots containing the inoculated plants in 3 to 4 in. of water. A rot of carrots caused by *P. megasperma* was found in Tasmania by White (4), but proof of pathogenicity was obtained only after the carrots were immersed in water for three days before inoculation.

All this evidence indicates that a wet soil condition is a predisposing factor for the infection of plants by *P. megasperma*.

It is thus obvious that good soil drainage is an important factor in the control of the foot rot disease of wallflower.

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A STUDY OF VARIABILITY IN CRESTED WHEATGRASS[1]

By R. P. Knowles[2]

Abstract

Thirty-one strains of crested wheatgrass from European and North American sources were assigned to six morphological forms. These forms showed affinities to the species *Agropyron cristatum* (L.) Gaertn., *A. desertorum* (Fisch.) Roem. & Schult., *A. sibiricum* (Willd.) P.B., *A. fragile* (Roth.) Nevski, *A. michnoi* Roshev., and *A. imbricatum* (M.B.) Roem. & Schult. Typical plants, spikes, glumes, and seeds of the six forms were illustrated. Eight strains of *A. cristatum* were diploid ($2n = 14$) and two strains were tetraploid ($2n = 28$). Strains of all other forms were tetraploid. Chromosome counts of root tips indicated no aneuploids in 71 diploid plants and one aneuploid ($2n = 29$) in 96 tetraploid plants. Meiotic observations, however, indicated accessory chromosomes in 14 of 43 tetraploid plants. Diploid forms of *A. cristatum* crossed sparingly with tetraploid species to form sterile hybrids. All tetraploid species were interfertile and formed fertile hybrids. Most desirable strains agronomically were of the species *A. cristatum*, *A. desertorum*, and *A. imbricatum*. Most vigorous hybrids were sterile plants from crosses of *A. desertorum* and *A. cristatum*.

Introduction

Crested wheatgrass was introduced to North America from Russia in 1906 (3). When distribution became extensive some 20 years later, the taller-growing form now recognized commercially as "Standard" was used in the United States. In Canada a shorter-growing form typified by the "Fairway" variety was more popular. Swallen and Rogler (18) classified the Standard and Fairway forms as *Agropyron desertorum* (Fisch.) Schult. and *A. cristatum* (L.) Gaertn. respectively.

Introductions of crested wheatgrass to the Forage Crops Laboratory, Saskatoon, conformed mainly to these two species but certain forms resembled additional species of crested wheatgrass described by Nevski (15). An attempt was made to assess the variability of forms of crested wheatgrass available to North American workers and find the interfertility of these forms. It was hoped that superior agronomic strains might be obtained by hybridization. Fairly extensive observations were made of strains to aid in further taxonomic treatment of this rather complex group of grasses.

Literature Review

Konstantinov (6) in 1923 concluded a critical analysis of crested wheatgrasses of the U.S.S.R. and found two fundamental forms. One form with wide spikes and short awns was recognized as *Agropyron cristatum* Bess. and the other with narrow spikes and reduced awns was classified as *A. desertorum* Fisch. There was much variation within these species and a large intermediate group was noted.

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Nevski (15) in 1934 described 13 forms of crested wheatgrass which he classified as separate species within the subgenus *Eu-Agropyron* of the genus *Agropyron*. The Fairway form cultivated in Canada, which has wide spikes, was classified as *A. pectiniforme* Roem. & Schult. rather than as *A. cristatum*. A strongly pubescent form with dense, purple-colored spikelets, collected in eastern Siberia and Mongolia, was regarded as *A. cristatum*. Forms similar to *A. pectiniforme* but with very pubescent lemmas and creeping rootstocks were designated as *A. michnoi* Roshev. Three species with narrow spikes were *A. desertorum* (Fisch.) Roem. & Schult., *A. sibiricum* (Willd.) P.B., and *A. fragile* (Roth.) Nevski. Species *A. sibiricum* and *A. fragile* were described as having very extended spikes with the latter species also having strongly pubescent leaves and leaf sheaths. Nevski considered *A. pectiniforme* and *A. desertorum* as the most valuable agronomic species because of their high yielding ability and drought resistance.

Subsequent reports by Sinskaja (17), Mashtakov (11), and Kosarev (8, 9) indicate an inclination by other Soviet workers to reduce the number of species from the number described by Nevski and to ascribe variation in form to ecological effects. Sinskaja assigned four of the wide-spiked species of Nevski to two main ecotype systems. The western system, extending from the lower Volga to the western Altai on moist and xerophytic habitats, was represented by the species *A. pectiniforme* and *A. imbricatum*. The eastern system, with mountain steppe and valley ecotypes from the eastern Altai and Mongolia, included the species *A. cristatum* and *A. michnoi* described by Nevski. Leaves in the eastern system were tougher and inclined to involution and hairiness which would denote adaptation to a more xerophytic habitat. Sinskaja regarded *A. imbricatum* and *A. michnoi* respectively as variants of *A. pectiniforme* and *A. cristatum* with pubescent spikes rather than as distinct species. The meadow steppe ecotype of the western ecotype system included tall, leafy, early types of high forage value.

Mashtakov (11) made a study of European and Asiatic forms of crested wheatgrass which represented six of the species described by Nevski. Most productive forms were those representing the species *A. imbricatum*, *A. pectiniforme*, and *A. desertorum* of Nevski. Mashtakov was inclined to agree with Konstantinov that only two species were represented in crested wheatgrass, that is, *A. cristatum* with wide spikes and *A. desertorum* with narrow spikes.

Kosarev (7) in 1941 listed four species of crested wheatgrass as being of agronomic value. These were *A. pectiniforme* and *A. cristatum* of the wide-spiked type, and *A. sibiricum* and *A. desertorum* of the narrow-spiked type. The pubescence of *A. imbricatum* was not considered by Kosarev as a satisfactory character for separating this form from *A. pectiniforme* as a separate species. The large number of intermediate forms indicated interpollination of forms, although the constancy of the four main types was maintained. Vladimirov (20) recorded the introduction of *A. imbricatum* into cultivation in 1950 and noted excellent seed production for this species under drought conditions in the Kuibishev province.

Cytological investigations in crested wheatgrass are not extensive and often confused by the uncertainty of taxonomic descriptions. Peto (16) found $2n$ chromosome numbers of 14 and 28 in introductions of *A. cristatum* from the U.S.S.R. Morphologically the 28-chromosome forms resembled *A. sibiricum* and *A. desertorum*, which species were found by Peto to have 28 chromosomes. The 14-chromosome group was considered as typical *A. cristatum*. The 28-chromosome forms were regarded as having originated from hybridization of 14-chromosome forms with a 42-chromosome *Agropyron* species such as *A. repens*, as reported by Troitsky (19).

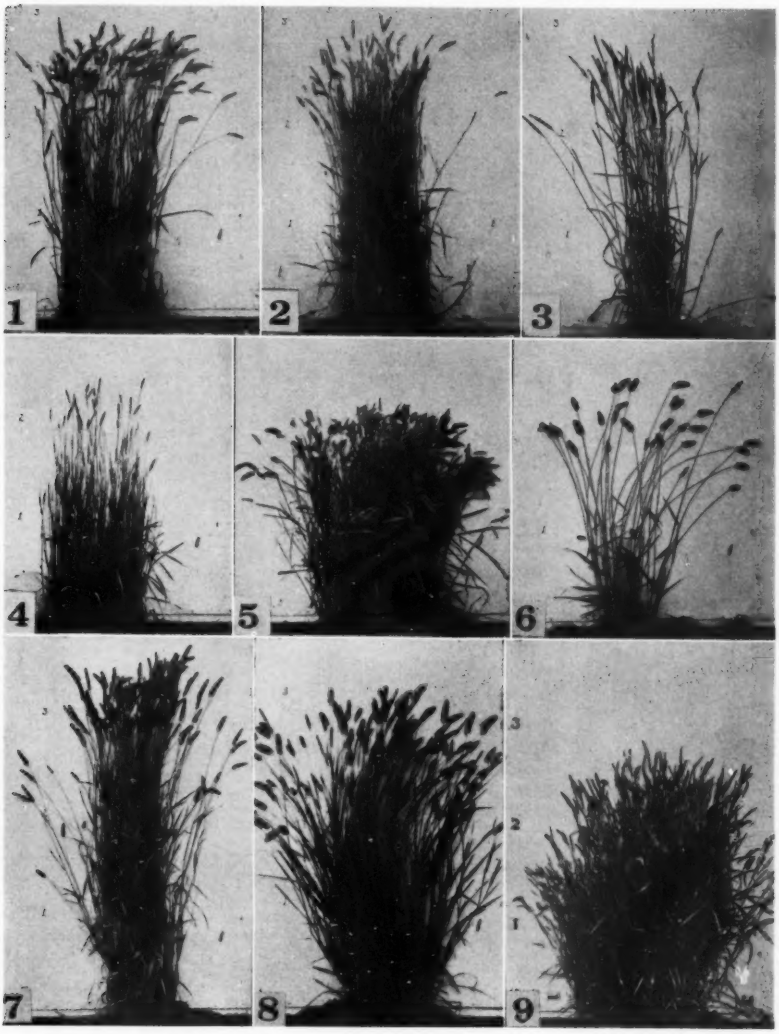
Avdulov (2) obtained somatic counts of 28 for both *A. cristatum* and *A. sibiricum*. Araratian (1) found chromosome numbers of 14, 28, and 42 in *A. cristatum* collections from Armenia and criticized the suggestion of Troitsky and Peto that 28-chromosome forms arose by the hybridization of *A. cristatum* and *A. repens*. Kotov (10), however, found forms resembling *A. sibiricum* and *A. fragile* in mixed populations of *A. pectiniforme* and *A. repens* which he considered evidence of hybridization of the last two species.

Dillman (3) reported *A. cristatum* and *A. desertorum* to have somatic chromosome counts of 14 and 28 respectively. Murphy (13) and Myers and Hill (14) also reported a chromosome number of 28 for the Standard type. Hartung (4) found a chromosome number of 28 for *A. sibiricum* and *A. michnoi*. For *A. cristatum*, Hartung reported a chromosome number of 14 for the Fairway strain and 28 for four other strains. Aneuploid plants with one to three extra chromosomes were observed by Peto, Myers and Hill, and Murphy. Myers and Hill observed an average quadrivalent frequency of 3.7 per pollen mother cell which suggested an autotetraploid derivation of the species.

Materials and Methods

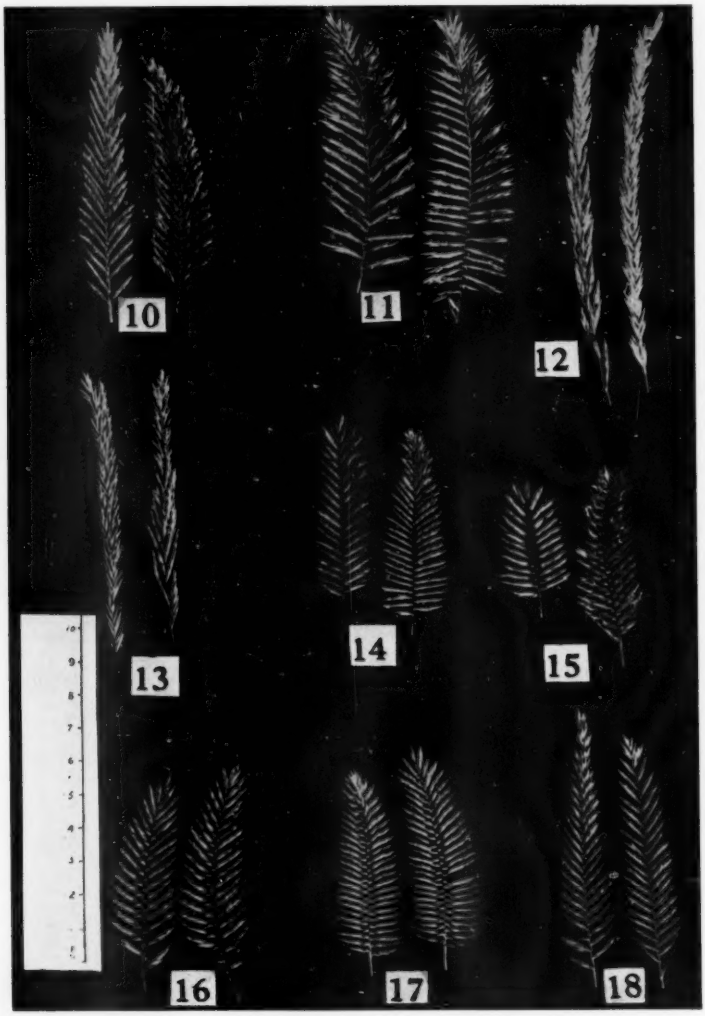
Strains used in these studies were obtained from the United States and various European countries including the U.S.S.R. When examined in spaced-plant nurseries six morphological forms were apparent. To facilitate discussion in this paper these six forms shall be referred to as separate species according to their similarities to the species *A. desertorum*, *A. sibiricum*, *A. michnoi*, *A. imbricatum*, and *A. fragile* described by Nevski (15). The Fairway form, placed in the species *A. pectiniforme* by Nevski, will be designated as *A. cristatum* (L.) Gaertn. to conform with general classification of this variety in North America (3, 18). Table I lists the strains used in this study with source and species designation. Table II gives morphological characteristics of material assigned to the six species. Figs. 1 to 24 present examples of plants, spikes, glumes, and seeds representative of the six forms.

Hybridization of plants was by bulk pollination without emasculation. Spikes of the seed parent were confined within parchment bags prior to flowering along with detached spikes of pollen parents. Detached spikes were kept fresh by maintaining their stems in vials of water. Single pollination bags were used for each cross with approximately five spikes of the seed parent enclosed in the crossing bag. As all plants were rather highly self-sterile the



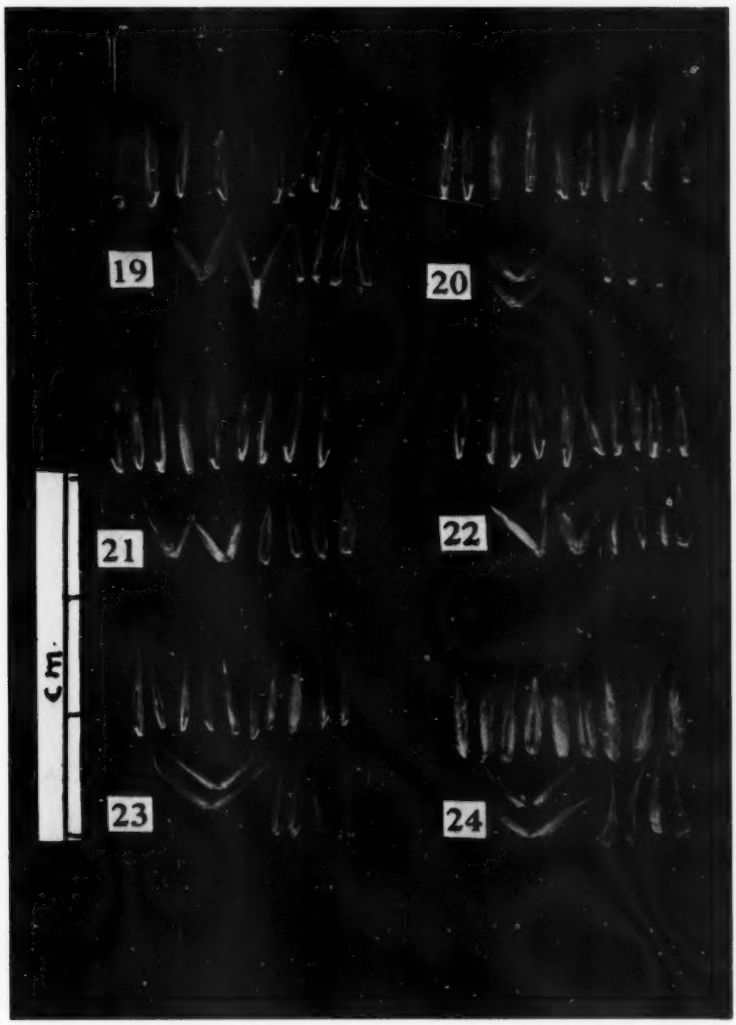
FIGS. 1–9. Plant habit observed in crested wheatgrass species and species hybrids.

FIG. 1. *A. desertorum* (S-1272).
FIG. 2. *A. imbricatum* (S-841).
FIG. 3. *A. sibiricum* (S-1334).
FIG. 4. *A. fragile* (S-1332).
FIG. 5. *A. cristatum* (Fairway).
FIG. 6. *A. michnoi* (S-294).
FIG. 7. *A. desertorum* × *A. michnoi*.
FIG. 8. *A. michnoi* × *A. cristatum*.
FIG. 9. *A. desertorum* × *A. cristatum*.



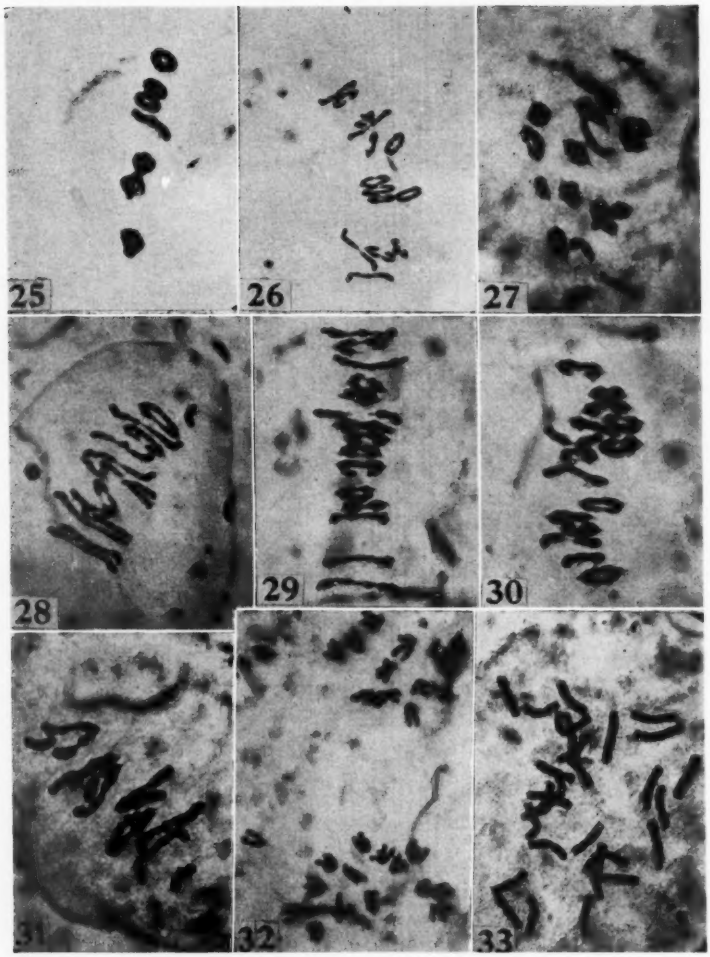
FIGS. 10–18. Spike types found in crested wheatgrass species and species hybrids.

FIG. 10. *A. desertorum* (S-1272).
FIG. 11. *A. imbricatum* (S-841).
FIG. 12. *A. sibiricum* (S-1334).
FIG. 13. *A. fragile* (S-1332).
FIG. 14. *A. cristatum* (Fairway).
FIG. 15. *A. michnoi* (S-294).
FIG. 16. *A. desertorum* × *A. michnoi*.
FIG. 17. *A. michnoi* × *A. cristatum*.
FIG. 18. *A. desertorum* × *A. cristatum*.



FIGS. 19–24. Seed and glume characteristics of crested wheatgrass species.

FIG. 19. *A. desertorum* (S-1272).
FIG. 20. *A. imbricatum* (S-2284).
FIG. 21. *A. sibiricum* (S-1334).
FIG. 22. *A. fragile* (S-1332).
FIG. 23. *A. cristatum* (Fairway).
FIG. 24. *A. michnoi* (S-294).



FIGS. 25–33. Meiosis and mitosis in crested wheatgrass species and species hybrids. (Magnification × 750).

FIG. 25. Metaphase *A. cristatum* (7 II).
FIG. 26. Metaphase *A. michnoi* (12 II, 1 IV).
FIG. 27. Metaphase *A. desertorum* (16 II, 1 small II).
FIG. 28. Metaphase *A. michnoi* × *A. imbricatum* (3 I, 11 II, 1 IV).
FIGS. 29, 30. Metaphase *A. michnoi* × *A. imbricatum* (15 II).
FIG. 31. Metaphase *A. desertorum* × *A. cristatum* (2 I, 2 II, 5 III).
FIG. 32. Anaphase *A. michnoi* × *A. sibiricum* (precocious division of dyad).
FIG. 33. Mitosis in root tip *A. desertorum* ($2n = 28$, same plant as found in Fig. 27).

TABLE I

STRAINS OF CRESTED WHEATGRASS USED IN HYBRIDIZATION AND CYTOLOGICAL STUDIES

Species	Strains and source
A. cristatum (L.) Gaertn.	Fairway variety (S.P.I. 19536) "Tall" selections (Fairway variety) S-1552 (Erevan, U.S.S.R.) S-1555 (Kompolt, Hungary) S-1566 (Gorki, U.S.S.R.) S-2657 (Krakow, Poland) S-2662 (Budapest, Hungary) S-3113 (Paris, France) S-3541 (Uppsala, Sweden) S-3543 (Baarn, Holland) S-3651 (Berlin, Germany)
A. imbricatum (M.B.) Roem. & Schult.	S-841 (Pullman, Wash., No. 3063-37) S-2284 (Moscow, U.S.S.R.) S-3652 (Paris, France)
A. michnoi Roshev.	S-294 (Omsk, U.S.S.R.) S-1515 (Mandan, N.D., P.I. 110152)
A. desertorum (Fisch.) Roem. & Schult.	Commercial (Saskatoon, Sask.) S-131 (Omsk, U.S.S.R.) S-1063 (U.S.D.A—W2412) S-1272 (Mandan, N.D., S.P.I. 19537) S-1289 (Bulgaria) S-1328 (U.S.D.A.—P.I. 111481) S-1333 (Logan, Utah, A1117) S-1556 (Sverdlovsk, U.S.S.R.) S-1580 (Alma Ata, U.S.S.R.) S-2259 (Alma Ata, U.S.S.R.) Nordan variety (Mandan, N.D.)
A. sibiricum (Willd.) P.B.	S-1298 (Kustanof, U.S.S.R.) S-1299 (U.S.D.A.—W-22) S-1334 (Logan, Utah, F.P.I. 108429)
A. fragile (Roth.) Nevski	S-1325 (Mandan, N.D., P.I. 108417) S-1332 (Logan, Utah)

seeds formed were generally the result of crossing. Hybrid plants were identified by the presence of characters of the pollen parent.

Chromosome doubling was attempted by treating coleoptiles of germinating seeds with lanolin containing 4% colchicine. Seedlings were treated when coleoptiles were $\frac{1}{4}$ in. long and planted one day after treatment.

Cytological examinations were made from temporary smears. A 3 : 1 alcohol – acetic acid mixture was used as a fixative for root tips and anthers. Root tips for somatic counts were collected from cuttings growing in troughs of running water. Excised root tips were pretreated with paradichlorobenzene for one-half to one hour prior to fixation. Aceto-orcein and acetocarmine stains were used for root tips and acetocarmine for pollen mother cells. Root tip counts generally were based on two clear figures for each plant with these in many cases coming from different tips.

TABLE II

MORPHOLOGICAL CHARACTERISTICS OF CRESTED WHEATGRASS STRAINS ASSIGNED TO DIFFERENT SPECIES

Character	*A. cristatum*	*A. imbricatum*	*A. michnoi*	*A. desertorum*	*A. sibiricum*	*A. fragile*
Root habit	Bunch	Bunch	Bunch-creeping	Bunch	Bunch	Bunch
Plant height, cm.	50–70	60–90	60–90	60–85	70–85	50–80
Culms per plant	Many	Intermediate	Few	Intermediate	Few	Intermediate
Leafiness	Good	Fair	Poor	Medium	Poor	Medium
Leaf pubescence	Upper surface	Nil	Nil	Nil	Nil	Upper and lower surfaces
Spike length, cm.	5–8	7–10	4–7	7–10	8–12	6–8
Spike width	Wide	Wide	Wide	Intermediate	Narrow	Narrow
Glumes	Divergent from lemma	Divergent from lemma	Divergent from lemma	Appressed to lemma	Appressed to lemma	Appressed to lemma
Lemma pubescence	Nil	Glabrous-pubescent	Dense pubescent	Glabrous	Glabrous	Glabrous
Awn length, mm.	2–6	0–10	2–4	0–5	0–2	0–1
Spikelet-bearing to rachis	Wide angle	Wide angle	Wide angle	Interm. angle	Narrow angle	Narrow angle
Color of leaves	Green-glaucous	Green	Glaucous	Green	Green	Green

Results

Chromosome Numbers of Crested Wheatgrass Species

Somatic chromosome numbers for plants of various strains are given in Table III. With the present range of strains it would appear that only diploid and tetraploid forms occur in crested wheatgrass. Tall robust plants suspected of being hexaploids or octoploids on the basis of spike and seed size were invariably found to be diploids or tetraploids. Strains listed as *A. cristatum* according to morphological characteristics were generally diploid, although two strains were tetraploid. These tetraploid strains were very similar to diploids in spike appearance but stems and leaves were coarser and seeds larger. Plants of *A. michnoi* were tetraploid although a single plant of the S-294 strain of *A. michnoi* was a triploid. As will be shown later this species intercrosses readily with *A. cristatum* to produce triploids. The very low frequency of aneuploid somatic counts is rather surprising in view of their observation by other workers in rather small populations (13, 14, 16).

TABLE III

SOMATIC CHROMOSOME NUMBERS FOR CRESTED WHEATGRASS SPECIES

Species	Strain	No. of plants	2*n* number
A. cristatum	Fairway	37	14
	"Tall" slection	15	14
	S-1552	3	14
	S-1555	3	14
	S-1566	4	14
	S-2657	2	14
	S-2662	1	14
	S-3113	3	28
	S-3541	3	14
	S-3543	1	28
	S-3651	3	14
A. imbricatum	S-841	5	28
	S-2284	11	28
	S-3652	3	28
A. michnoi	S-294	1	21
	S-294	7	28
	S-1515	3	28
A. desertorum	Commercial	7	28
	S-131	14	28
	S-1063	5	28
	S-1272	11	28
	S-1289	2	28
	S-1328	1	28
	S-1333	3	28
	S-1556	3	28
	S-1580	1	28
	S-2259	2	28, 29
	Nordan	3	28
A. sibiricum	S-1298	2	28
	S-1299	5	28
A. fragile	S-1325	2	28
	S-1332	2	28

Table IV indicates chromosome associations observed at first metaphase division of pollen mother cells. Seven bivalents were formed regularly in the diploid form of *A. cristatum* (Fig. 25). Tetraploid species showed considerable irregularity with the production of univalents, trivalents and higher associations. Generally in tetraploid species there were approximately 10 bivalents and from one to three quadrivalents. Greatest regularity in the formation of bivalent or quadrivalent associations for tetraploid species was in the S-2284 strain of *A. imbricatum* and the S-294 strain of *A. michnoi* (Fig. 26). The average number of quadrivalent associations in *A. desertorum* was considerably lower than the average of 3.7 noted by Myers and Hill for this species (14).

Accessory chromosomes were observed in PMC's (pollen mother cells) of 14 of the 43 tetraploid plants observed at meiosis (Fig. 27). These accessories were frequently fragments or univalents but also included normal appearing chromosomes which paired at meiosis. In contrast to the frequent occurrence of accessory chromosomes at meiosis of tetraploid plants, they were rarely observed in root tips (cf. Figs. 27 and 33). Only one plant in 96 tetraploids was found to have a single additional chromosome in somatic counts. Accessory chromosomes at meiosis were not seen in plants of *A. sibiricum* or

TABLE IV

CHROMOSOME ASSOCIATIONS AT FIRST MEIOTIC METAPHASE IN POLLEN MOTHER CELLS OF CRESTED WHEATGRASS SPECIES

Species and strain	2*n* No.	No. of plants	No. of PMC's	Av. frequency of associations				
				I	II	III	IV	V
A. cristatum								
Fairway	14	13	130		7.0			
A. imbricatum								
S-841	28	1	20	0.4	8.2	0.2	2.7	
"	29	1	3	0.5	9.5	0.5	2.0	
"	30	2	12	1.3	10.8	0.1	1.6	
"	31	1	7	1.1	9.6	0.3	2.3	0.1
"	34	1	7	2.0	10.6	0.2	2.4	0.1
S-2284	28	6	57		10.2		1.9	
A. michnoi								
S-294	28	10	91	0.1	8.8		2.6	
"	29	1	19	1.0	10.5	0.1	1.6	0.1
A. desertorum								
All strains	28	6	58	0.3	9.1	0.2	2.2	
	29	4	38	1.0	10.3	0.1	1.6	0.1
	30	3	21	0.1	9.3	0.3	2.4	0.1
	32	1	11	0.4	13.4		1.2	
A. sibiricum								
All strains	28	4	26	0.8	10.0		1.8	
A. fragile								
All strains	28	2	10	0.1	8.5	0.2	2.5	

A. fragile but only small numbers of plants in these two species were examined. Diploid strains of *A. cristatum* did not show supernumerary chromosomes. Preliminary observations indicated no relationship between the occurrence of supernumeraries and the vigor of plants.

Interfertility of Species

Three plants of each of the six species were pollinated with three plants of each of the other species. Crossing was facilitated by arranging three groups of plants with each group containing a single plant of each species. Reciprocal pollinations were made between all plants within each group. Although *A. imbricatum* and *A. michnoi* species flowered a few days earlier than other species and *A. fragile* a few days later, it was possible to select plants within these species which flowered on the same days as the remaining species. Pollination bags were examined to check coincidence of flowering. Certain plants were slightly self-fertile so that progenies grown from seed produced in pollination bags contained a few self plants as well as hybrids. Table V indicates the number of hybrid plants which could be identified in progenies by the presence of characters of the pollen parent.

The species *A. cristatum* was represented by diploid plants of the Fairway variety and strain S-1566. These strains did not cross readily with any tetraploid species except *A. michnoi*. This cross occurred quite freely as shown by the appearance of triploid plants in open-pollinated progenies of *A. michnoi*. A few hybrid plants were identified in progenies of *A. cristatum* when

TABLE V

NUMBER AND VIGOR OF F_1 HYBRID PLANTS RESULTING FROM THE INTERPOLLINATION OF CRESTED WHEATGRASS SPECIES

Plants rated 1 (low) to 10 (high) for vigor with Fairway check rated 7.0

Seed parent		Pollen parent					
		A.c.	*A.i.*	*A.m.*	*A.d.*	*A.s.*	*A.f.*
A. cristatum (*A.c.*)	No. of plants	—	2	14	3	4	0
	Vigor score	—	4.0	6.4	6.3	5.0	—
A. imbricatum (*A.i.*)	No. of plants	0	—	0	15	4	21
	Vigor score	—	—	—	6.3	5.5	5.1
A. michnoi (*A.m.*)	No. of plants	4	13	—	17	9	14
	Vigor score	5.5	6.5	—	6.0	6.0	5.6
A. desertorum (*A.d.*)	No. of plants	0	19	0	—	16	0
	Vigor score	—	6.6	—	—	5.5	—
A. sibiricum (*A.s.*)	No. of plants	0	10	3	8	—	14
	Vigor score	—	6.6	4.7	6.0	—	5.1
A. fragile (*A.f.*)	No. of plants	0	3	4	5	6	—
	Vigor score	—	6.0	4.5	2.4	3.7	—

A. imbricatum, *A. desertorum*, or *A. sibiricum* was the pollen parent but not when *A. fragile* was the pollen source. In view of the desirability of obtaining crosses of *A. cristatum* and *A. desertorum* several crosses were attempted in addition to those indicated above but only a small number of hybrid plants were obtained. When used as a pollen parent, *A. cristatum* crossed with *A. michnoi* but not with other tetraploid species. There appeared to be considerable interfertility of tetraploid species, although *A. michnoi* did not cross as a pollen parent with *A. imbricatum* or *A. desertorum*.

Hybrids of Crested Wheatgrass Species

Hybrid plants were examined for desirable combinations of parental characteristics such as the good leafiness of the Fairway variety and the tall growth habit of tetraploid species. All plants were scored on the basis of 1 (low) to 10 (high) for vigor. Figs. 7, 8, and 9 show some of the more promising F_1 hybrid plants produced. Hybrids of *A. desertorum* and *A. michnoi* were quite tall and aggressive but inclined to be coarse and lacking in leafiness. Sixty-three hybrid plants of this cross were given an average score of 5.9 as compared to a score of 7.0 for the Fairway variety. Sixteen of these hybrids were sterile. Triploid crosses resulting from the hybridization of *A. michnoi* and *A. cristatum* were generally vigorous but again showed the stemmy nature of the *A. michnoi* parent. Thirty hybrid plants of this cross were given an average vigor score of 6.1 compared to a score of 7.0 for Fairway plants. All hybrid plants of this cross were rather highly sterile. An interesting feature of these sterile plants was the delayed ripening off of stems and leaves which is desirable from a forage quality standpoint. Hybridization of *A. desertorum* and *A. cristatum* gave sterile hybrids with great variability in leafiness and vigor. This hybrid appears the most promising of all hybrids providing fertility can be obtained through doubling with colchicine or further hybridization.

Chromosome doubling by the use of colchicine was successful in the triploid cross of *A. michnoi* × *A. cristatum*. Only three triploid plants were doubled and as isolation was not provided these plants crossed with surrounding plants in the nursery to produce pentaploids ($2n = 35$). These pentaploids showed certain spike characteristics of *A. desertorum* and presumably were trihybrids of the constitution (*A. michnoi* × *A. cristatum*) × *A. desertorum*. Triploid hybrids of the cross *A. desertorum* × *A. cristatum* were treated with colchicine to obtain hexaploids but thus far no doubled sectors have been identified.

Table VI presents a summary of chromosome associations observed at meiosis in F_1 hybrid plants. The occurrence of supernumerary chromosomes again made interpretation of associations difficult. In one plant of the cross *A. imbricatum* × *A. michnoi* a variation in chromosome number from 28 to 30 also was observed at meiosis. The frequencies of bivalent and quadrivalent associations in tetraploid crosses were fairly comparable to those in parent species. However, crosses of *A. michnoi* and *A. desertorum* showed higher bivalent and lower quadrivalent frequencies than found in either of the parent

TABLE VI

MEIOTIC ASSOCIATIONS IN F_1 HYBRID PLANTS OF CRESTED WHEATGRASS SPECIES

Cross	2n No.	No. of plants	No. of PMC's	I	II	III	IV	V	VI	VII	VIII
Crosses of diploids and tetraploids											
A. michnoi, *A. cristatum*	21	8	79	5.3	5.4	1.5					
" "	22	1	10	4.4	6.2	1.6					
" "	23	1	10	6.0	6.3	1.3		0.1			
A. desertorum, "	21	5	55	3.0	3.6	3.2	0.2				
A. sibiricum, "	21	1	10	2.2	3.1	3.2	0.5	0.2			
Crosses of tetraploids											
A. imbricatum, *A. desertorum*	30	1	5	0.4	11.2		1.8				
" , *A. sibiricum*	28	1	6	1.8	10.8	0.8	0.5				
" , *A. fragile*	28	1	5	0.8	9.0		2.0		0.2		
" , *A. michnoi*	29	1	10	2.9	10.2	1.1	0.6				
" , "	28–30	1	10	0.6	13.8	0.1	0.2				
" , "	30	1	10	0.2	13.7		0 6				
A. michnoi , *A. desertorum*	28	5	68	0.1	12.3		0.8				
" , "	29	2	14	0.9	9.9	0.4	1.4	0.1	0.1		
" , *A. sibiricum*	28	4	40	0.5	11.0	0.1	1.3				
" , "	30	1	10		11.8		1.6				
" , *A. fragile*	28	2	20		11.5		1.2				
A. desertorum, *A. sibiricum*	28	1	10	0.2	7.0	0.2	2.5	0.2	0.1	0.2	
A. sibiricum, *A. fragile*	32	1	5	0.4	10.8		1.8		0.2		0.2

species. This might indicate some lack of homology of *A. michnoi* and *A. desertorum* chromosomes. A relatively low frequency of trivalents was observed in crosses of *A. michnoi* and *A. cristatum* and a relatively high frequency of trivalents in crosses of *A. cristatum* with *A. desertorum* and *A. sibiricum*. More complete trivalent association would be expected in crosses of *A. cristatum* with *A. desertorum* and *A. sibiricum* on the basis of similarity of plant type. Ease of crossing, however, indicated a closer relationship between *A. cristatum* and *A. michnoi* than between *A. cristatum* and *A. desertorum*.

Discussion

It is not possible with the present limited material and the lack of type specimens to make conclusions regarding a suitable taxonomic treatment in the crested wheatgrasses. The present study does, however, give an indication of the variability in the crested wheatgrass complex and the interfertility of various morphological forms.

Until recently both the Fairway and Standard forms of crested wheatgrass were classified in North America as *A. cristatum*. Dillman (3) suggested that Standard strains be recognized as *A. desertorum* and Fairway strains as *A. cristatum*. Swallen and Rogler (18) also recognized the Standard varieties as *A. desertorum* but found certain broad-spiked forms within the Standard type which they classified as *A. cristatum*. They suggested that the uniformity and constancy of the Fairway variety warranted a separate species classification but temporarily included this variety in *A. cristatum*.

The present study indicates that the Fairway variety and several similar strains are diploid and do not cross readily with tetraploid forms. It would appear that the diploid form should have a separate designation, possibly *A. cristatum* or *A. pectiniforme*. Tetraploid forms referred to in this paper as *A. imbricatum* appear similar to broad-spiked forms of the Standard material which Swallen and Rogler classed as *A. cristatum*. Since this form resembles *A. desertorum* in leaf and stem characteristics and intercrosses readily with *A. desertorum* to produce fully fertile hybrids, classification as an independent species does not seem warranted.

Hybrids of diploid *A. cristatum* and tetraploid *A. desertorum* showed a high frequency of trivalents at meiosis. This indicates considerable homology of chromosomes of the diploid type and of *A. desertorum*. High trivalent frequency also indicates that *A. desertorum* is autoploid in origin or an alloploid of the diploid form used in these studies and a closely related diploid form. Introduced strains were observed closely for a slender form of the Standard type which might be diploid, but none was found.

Forms referred to as *A. sibiricum*, *A. fragile*, and *A. michnoi* had the $2n$ chromosome number of 28 and crossed with *A. desertorum* to give fertile hybrids. However, these three forms appear morphologically and to some extent physiologically distinct. Most Soviet workers have stressed the adaptation of these species to sandy soils and severe climatic conditions (6, 7, 15). The S-294 strain listed as *A. michnoi* and the strains of *A. fragile* possessed very distinctive leaf and spike characteristics which could prove useful in genetic studies and tests of the distance of pollen dispersal.

The maintenance of tetraploid forms in mixed nursery plantings for several generations is difficult because of the interfertility of these forms and the production of fertile crosses. Differences in flowering dates appear the main factor in maintaining purity of the various forms. The *A. michnoi* form flowered two to three days earlier than *A. desertorum* and at somewhat lower temperatures. Strains of *A. imbricatum* also flowered two to four days in advance of *A. desertorum*. Plants of *A. sibiricum* and *A. fragile* flowered one to two days later than *A. desertorum*. The peak of flowering of *A. desertorum* generally preceded the flowering in *A. cristatum* by one day but there was considerable overlapping of flowering.

Hybridization of these last two species is prevented by the ploidy barrier and the fact that the few hybrids found are sterile. It was noted, however, that two of the three crosses *A. cristatum* × *A. desertorum* were fertile which would indicate that unreduced gametes of *A. cristatum* occasionally unite with normal gametes of *A. desertorum* to produce fertile plants.

Agronomically *A. cristatum* (Fairway variety only), *A. desertorum*, and *A. imbricatum* appear the most useful species. Numerous strain trials in Western Canada have shown strains of *A. desertorum* to excel the Fairway variety slightly in hay yields particularly under favorable moisture conditions. Strains S-841 and S-2284 of *A. imbricatum* have shown forage yields comparable with better strains of *A. desertorum*. At Saskatoon the forage

yields of S-1298 and S-1299 strains of *A. sibiricum* were 85 and 65% respectively of that of the Fairway variety. Unfortunately this species has not been tested under sandy soil conditions in Canada.

Supernumerary chromosomes not homologous with normal chromosomes have been noted in several plant species, including grasses. These supernumerary chromosomes or *B* chromosomes characteristically show no pairing with normal chromosomes although they frequently pair among themselves. Generally they are considered genetically inert. Supernumeraries noted in crested wheatgrass resemble those reported as occurring in *Sorghum purpureosericeum* (5) and *Poa alpina* (12) in that they appear rather frequently at meiosis but rarely in root-tip mitosis. They were more frequent in some tetraploid forms of crested wheatgrass than in others, but the variation between forms did not appear to be due to their presence. Supernumeraries could not be identified by the staining reaction. In certain plants they appeared smaller than normal chromosomes (Fig. 27) but frequently they could not be distinguished from other chromosomes at meiosis on the basis of size (Figs. 29, 30). The fact that supernumerary chromosomes were found in tetraploid and not in diploid plants would suggest that their origin and maintenance might be related to the more complex pairing relationships during meiosis in tetraploids. Fig. 32 shows a type of precocious division of dyads which would give rise to pollen grains with additional chromosomes. Since crosses of *A. michnoi* and *A. cristatum* showed supernumeraries regardless of the direction of the cross, it would appear that supernumeraries were transmitted through both the pollen and the egg.

Acknowledgments

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WINTER RESTING STAGES OF CERTAIN ERICACEAE[1]

By Hugh P. Bell[2] and Jane Burchill[3]

Abstract

The winter resting stages of 17 species of Ericaceae are described, figured, and analyzed. From this analysis, the following deductions were made: (1) Each species has a fairly definite winter resting stage. (2) As rates of development vary from species to species, the winter resting stage is not in all cases related to the time of full bloom. (3) The embryo sac and anther do not develop at similar rates; hence, for any stage in the development of one, there is not a definite accompanying stage in the development of the other. (4) The Ericaceae were not evolved in a climate which had the marked periodicity and extreme cold characteristic of our present north temperate zone.

Introduction

This study of the winter resting stages of certain Ericaceae was an attempt to find answers to the following questions: (1) Is there for each species a definite resting stage that does not vary from season to season or from locality to locality? (2) Does the winter stage have any relation to the time of full bloom? (3) For each stage in the development of the embryo sac or anther, is there a definite accompanying stage in the development of the other? (4) Do the winter resting stages of these Ericaceae lend support to the theory of plant geographers that this family was evolved in a region where it was possible for the plants to bloom for most of the year without any definite resting period?

Materials and Methods

All the winter buds were collected in Nova Scotia and most of them from plants growing in the vicinity of Halifax. For the species from four genera, namely, *Chamaedaphne*, *Epigaea*, *Ledum*, and *Rhododendron*, the collections were started in 1913 and have been continued at intervals during the intervening years. For *Vaccinium angustifolium* collections have been made regularly over a period of 12 years, and during one year they were made at varied and widely separated localities in Nova Scotia (2). For most of the other species the period during which collections were made was more than two years. For some species the winter bud alone was not sufficient to indicate the exact stage in which the plant overwintered. In all these cases collections were continued until after growth started in the spring. Then after studying this later growth, it was always possible to interpret the sections of the winter buds.

To indicate the suitability of these buds for the study of winter resting stages, some information should be provided regarding the winter conditions to which they were subjected. As reported by Hornstein (9) for the Halifax

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area from 1945 to 1954 inclusive, the average monthly means were: November 42.07° F., December 32.20° F., January 27.06° F., February 26.57° F., and March 32.85° F. For the same period, the average monthly minimums were: November 24.01° F., December 8.95° F., January −0.14° F., February 2.77° F., and March 10.90° F. From these data it is evident that, for at least the months of January and February, the temperatures are well below that at which growth occurs in the average land plant. That is, although the temperatures are not extremely low, they are low enough to bring on a period of at least partial dormancy in the flora of the district.

The most satisfactory killing fluid was found to be Nawaschin's, and the most satisfactory stain, iron alum hematoxylin.

Species Studied

The species studied were the more common natives of Nova Scotia and included *Andromeda glaucophylla* Link, *Arctostaphylos Uva-ursi* (L.) Spreng. var. *coactilis* Fern. & Macbr., *Chamaedaphne calyculata* (L.) Moench. var. *angustifolia* (Ait.) Rehd., *Epigaea repens* L. var. *glabrifolia* Fern., *Gaultheria hispidula* (L.) Bigel., *Gaultheria procumbens* L., *Gaylussacia baccata* (Wang.) K. Koch, *G. dumosa* (Andr.) T. & G., *Kalmia angustifolia* L., *K. polifolia* Wang., *Ledum groenlandicum* Oeder., *Rhododendron canadense* (L.), Torr., *Vaccinium angustifolium* Ait. var. *laevifolium* House., *V. macrocarpon* Ait., *V. myrtilloides* Michx., *V. Oxycoccus* L., *V. Vitis-Idaea* L. var. *minus* Lodd. The names used are those to be found in the latest edition of Gray's Manual (7). The details regarding the resting stage for each of these will now be given, but as this study is concerned solely with the reproductive tissues, these are the only parts of the florets or buds which will be described. For ease of reference the species are arranged in alphabetical order.

Andromeda glaucophylla

The ovule is just starting to curve, the one layer of nucellar cells is distinct, and there may be two archesporial cells, the outer one enlarging. There is no sign of an integument (Fig. 1).

The stamen has its mature shape and orientation. In the anther all layers are distinct. The middle wall cells are elongated, but neither they nor the tapetum are disintegrated in any way. In the sporogenous tissue the cells are sharply differentiated but are comparatively small (Fig. 2). When growth starts in the spring they enlarge by about twenty-five per cent in all directions until they attain the size of mature microspore mother cells. No additional mitosis was observed before the initiation of meiosis.

Arctostaphylos Uva-ursi

The ovules are pendant from the upper edge of a central receptacular elevation. There is no archesporial cell and no integument (Fig. 3). The archesporial cell is formed about the middle of April, shortly after the start of spring growth. The integument is formed still later.

In the anthers the sporogenous tissue is sharply differentiated, but it has not reached the microspore mother cell stage for two cases of mitosis were observed in buds collected on December 23rd. In the walls tapetum differentiation is just starting, and there are usually two middle layers of living cells (Fig. 4).

Chamaedaphne calyculata

The female gametophyte is in the two nucleate stage, and the nucellar cells have completely disintegrated (Fig. 5). The integument is thick, and the micropyle is distinct. This is the same as Palser's Fig. 628 for this species (12). In all our collections this was the condition found as the winter stage, and these collections were started in 1914 and have been continued at intervals since then. On this evidence one would be inclined to regard this as fairly constant for the species, but Palser (12, p. 42) reports finding the "functional megaspore stage" during March in material collected in southern Michigan; so apparently the winter stage is not so constant as the Nova Scotian material would suggest.

The anther is completely developed. The cell contents of the middle wall layers and of the tapetum have disintegrated. The tapetal cells do not stain deeply. The pollen grains are binucleate and in tetrads (Fig. 6).

Epigaea repens

This flower is mature and ready to open.

In the embryo sac there are two synergids, an egg cell, two polar nuclei, and three degenerating antipodals. The three antipodals may be in a row but are usually arranged at random at the chalazal end of the embryo sac. The only change which may occur between the winter stage and the time of blossoming is the complete degeneration of the antipodals, for Palser (12, p. 44) reports that there are no antipodals in the embryo sac of the mature flower. The embryo sac is surrounded by a thick integument (Fig. 8).

As previously reported (3), mitosis was observed in flower buds collected on January 5, 1953, when the temperature was about 26° F. This mitosis was taking place in the embryo sac, which was probably in the four nucleate stage of the gametophyte. Since the time when this was observed, winter collections have been made in the same locality and during or following periods of warm weather. On examining these it was found that all the embryo sacs were mature and no mitotic figures could be found.

The anthers may dehisce during the winter while the flower is still tightly closed (Fig. 9). The pollen grains are binucleate and in tetrads. The middle wall layers are crushed and the cell contents of the tapetum and of the wall layers have disintegrated (Fig. 7).

With this flower in the mature condition described above it is not surprising that during an exceptionally warm period the flowers may open in the late autumn or winter. For instance, the authors collected fully opened flowers on December 29, 1954.

Gaultheria hispidula

The ovule has turned completely into its anatropous position. A distinct integument has developed and may extend to a position about half way towards the tip of the nucellus. The archesporial tissue is in the megaspore mother cell stage remaining unchanged until the initiation of meiosis in the spring (Fig. 10). Sometimes there are two archesporial cells.

In the anther the sporogenous tissue is sharply differentiated, but it is not made up altogether of spore mother cells, for in the buds collected after the start of spring growth mitosis was taking place in a number of the cells. The tapetum is differentiated, but not sharply so, for in places it is difficult to distinguish between the tapetal cells and those of the middle wall layers. The cells of these middle wall layers are living and are usually elongated but not crushed (Fig. 11).

Gaultheria procumbens

As reported by Chou (5, p. 205) "Flowers are initiated on the new growth of the current year. . .". The meristematic tissue, which will form this new growth, overwinters as a purely vegetative bud on the rhizome (Fig. 14).

Gaylussacia baccata

In the ovary the placentae are represented by 10 oval mounds of meristematic tissue but the ovule primordia are not differentiated (Fig. 13). These ovule primordia appear early in the spring as basipetally directed outgrowths from the lower region of each placenta.

In the stamen the anther is inverted but in most cases there is very little differentiation of sporogenous tissue. However, in the more basipetally placed florets the four regions may be defined (Fig. 12*a*), but even then there is no sign of a tapetum, and there are at the most two wall layers of living undifferentiated cells (Fig. 12*b*). This is the most advanced condition for the anther in this flower, for proceeding acropetally there is every gradation to anthers in which there is no differentiation of sporogenous tissue. Intermediate cases were observed where there were a few sporogenous cells at one end of the anther and none at the other end.

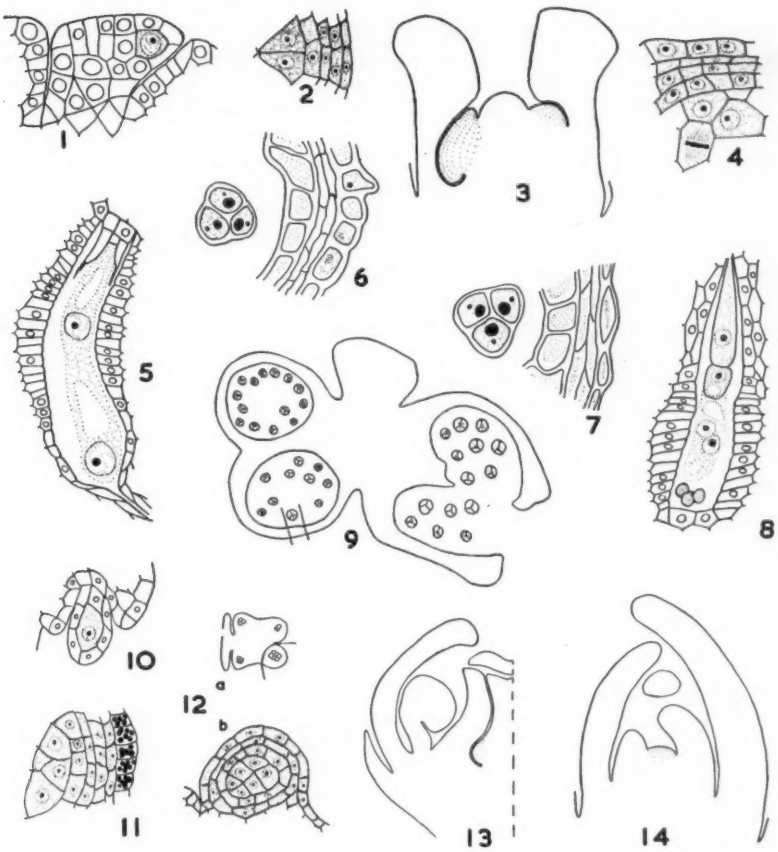
Gaylussacia dumosa

There is no sign of carpel primordia. The differentiation of embryonic bracts, sepals, and petals is well advanced but the differentiation of the stamen primordia is just starting. These structures surround a central mound of undifferentiated meristematic tissue (Fig. 15).

Kalmia angustifolia

The main floral parts are differentiated but there is no differentiation of reproductive tissue in either the carpel or the stamen.

In the pistil the future ovary is slightly swollen. There are minute placental primordia on the margins of each carpel but they are in such an early



FIGS. 1 and 2. *Andromeda glaucophylla*. FIG. 1. Ovule in megaspore mother cell stage (× 360). FIG. 2. Section of anther wall and two sporogenous cells (× 360).

FIGS. 3 and 4. *Arctostaphylos Uva-ursi*. FIG. 3. L.s. (longitudinal section) of floret (× 85). FIG. 4. Section of anther wall and three sporogenous cells, one of them in metaphase (× 360).

FIGS. 5 and 6. *Chamaedaphne calyculata*. FIG. 5. Female gametophyte in two nucleate stage (× 360). FIG. 6. Section of anther wall and one tetrad with binucleate pollen grains (× 360).

FIGS. 7 to 9. *Epigaea repens*. FIG. 7. Section of anther wall and one tetrad with binucleate pollen grains (× 360). FIG. 8. Mature female gametophyte. One synergid is below the plane of the drawing (× 360). FIG. 9. T.s. (transverse section) of anther, one loculus dehiscing (× 85).

FIGS. 10 and 11. *Gaultheria hispidula*. FIG. 10. Ovule in megaspore mother cell stage (× 360). FIG. 11. Section of anther wall with sporogenous cells (× 360).

FIGS. 12*a* to 13. *Gaylussacia baccata*. FIG. 12*a*. Diagram of t.s. of anther (× 85). FIG. 12*b*. Early stage of sporogenous tissue and wall layers (× 360). FIG. 13. L.s. half of floret. Placenta primordium stippled (× 85).

FIG. 14. *Gaultheria procumbens*. Flowering branch primordium (× 85).

stage of development that at times it is difficult to locate them (Fig. 16 and Fig. 17). The future loculi are merely crescent shaped slits at the outer ends of five very narrow radially arranged spaces which extend from the stylar canal to the peripheral region of the carpel primordia (Fig. 16).

The stamen primordia are swollen at their distal end but both the future anther and the future filament are mostly meristematic tissue (Fig. 17).

Kalmia polifolia

The ovule is complete with a fully formed integument. The female gametophyte is in the two nucleate stage, and is surrounded by the disintegrated cells of the nucellus (Fig. 18).

In the anther the cell contents of the tapetum and of the middle wall layers have disintegrated. The pollen grains are uninucleate and in tetrads (Fig. 19).

Ledum groenlandicum

In the carpels the ovule primordia are only slightly curved and there is no development of archesporial tissue or integument (Fig. 20).

The anther is at about the same stage of development as that of *Kalmia polifolia*. That is, the cell contents of the middle wall layers and of the tapetum have disintegrated. The pollen grains are uninucleate and in tetrads (Fig. 21).

If the winter stage of this species is compared with that of others such as *Vaccinium angustifolium* or *Gaultheria hispidula*, it is apparent that the ovules of this *Ledum* are in a very early stage whereas its anthers are decidedly advanced.

Rhododendron canadense

The ovules of this species are different from those of the other forms included in this study in that the archesporial cells vary in number from one to as many as seven. The one illustrated in Fig. 22 is typical of many observed. In the winter material it was not always possible to determine which of the many archesporial cells would become functional. To check this point material was collected three months later just as spring growth was starting. In this early spring material the outermost cell was always the largest of the row; hence it would appear that it is the one which becomes the functional megaspore mother cell. The integument in this winter stage is represented by merely a slight enlargement at the base of the ovule (Fig. 22).

The stage of development of the anthers is similar to those of *Kalmia polifolia* and *Ledum groenlandicum*. That is, the middle wall layers are crushed and the cell contents of the middle wall layers and of the tapetum are partially disintegrated. The pollen grains are uninucleate and in tetrads (Fig. 23).

Vaccinium angustifolium

The winter stage of this species has already been described in detail (2). Briefly it is as follows: in the basipetally placed florets, the ovule is curved.

The integument has developed to a position half way to the tip of the nucellus and the archesporial cell may have assumed the shape of a typical megaspore mother cell. There may be two archesporial cells. As a rule the ovule is similar to that of *Gaultheria hispidula* as illustrated in Fig. 10.

In the stamen the sporogenous tissue is in the microspore mother cell stage. The tapetum and middle wall layers are completely differentiated and are not crushed. This is similar to the condition in *Vaccinium myrtilloides* as illustrated in Fig. 27. As reported previously (3) mitosis was observed in winter collections of *V. angustifolium*.

Vaccinium macrocarpon

In the basipetally placed florets the pistil primordium is a flouted ring of tissue surrounding a central depression. Immediately outside this ring are eight stamen primordia which are merely slightly club-shaped mounds of meristematic tissue. The bases of these stamen primordia are slightly above the lowest point of the central depression and their apices are above the distal margin of the pistil primordium (Figs. 24 and 25). The more apically placed florets are much smaller and in an earlier stage of development.

Vaccinium myrtilloides

The curvature of the ovule is complete and there may be more than one archesporial cell. The integument is not differentiated (Fig. 26).

The stamen is similar to that of *V. angustifolium*, that is, the sporogenous tissue is in the microspore mother cell stage; the parietal layers are differentiated and living (Fig. 27).

This species is the only case in this study where the description of the winter stage is based on the buds from only one clone. The plants from this clone were very carefully identified during the summer, but the lack of other collections was not noted until it was too late to remedy the situation. For in the vicinity of Halifax there is a wide variation in the plants usually assigned to this species. Therefore careful identification must be made during the summer before one can be sure of having a typical representative.

Vaccinium Oxycoccus

The apical floret primordia are just elongated mounds of meristematic tissue (Fig. 28).

The basipetally placed florets have two bracts and the primordia of sepals and petals. In the center where the primordia of the stamens and carpels will be formed there is merely an undifferentiated mound of meristematic tissue (Fig. 29).

Vaccinium Vitis-Idaea

The four placentae are differentiated as mounds of meristematic tissue and the start of ovule primordia formation is indicated by the presence of barely perceptable undulations on the outer surface of these mounds (Fig. 30).

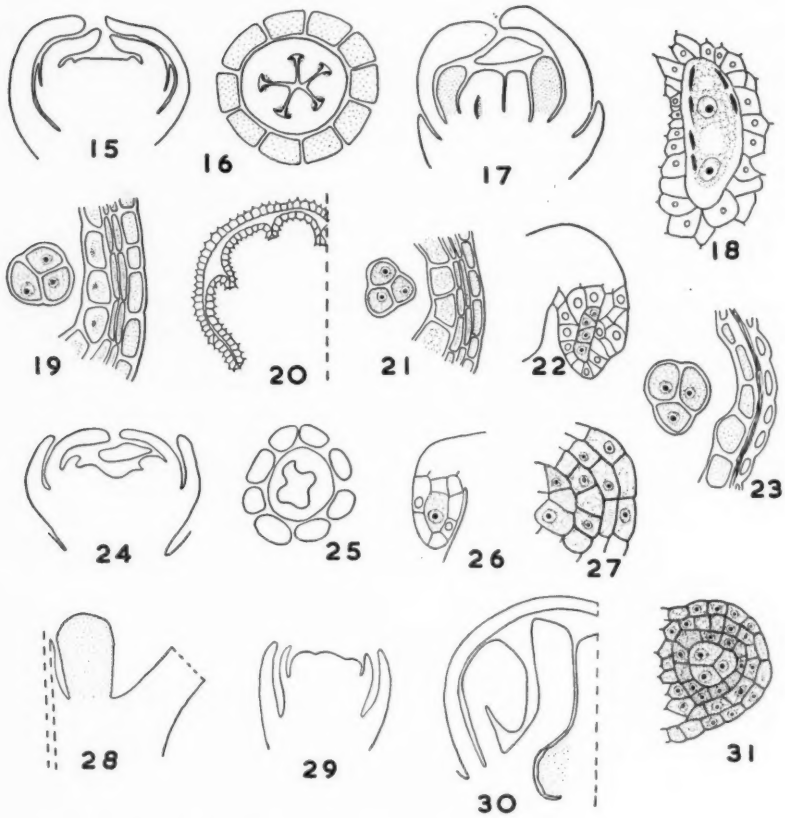


FIG. 15. *Gaylussacia dumosa*. L.s. floret (× 85).

FIGS. 16 and 17. *Kalmia angustifolia*. FIG. 16. T.s. floret. Ovary and stamens only (× 85). FIG. 17. L.s. floret (× 85).

FIGS. 18 and 19. *Kalmia polifolia*. FIG. 18. Female gametophyte in two nucleate stage (× 360). FIG. 19. Section of anther wall and one tetrad with uninucleate pollen grains (× 360).

FIGS. 20 and 21. *Ledum groenlandicum*. FIG. 20. T.s. half of loculus (× 225). FIG 21. Section of anther wall and one tetrad with uninucleate pollen grains (× 360).

FIGS. 22 and 23. *Rhododendron canadense*. FIG. 22. Ovule with four archesporial cells (× 360). FIG. 23. Section of anther wall and one tetrad with uninucleate pollen grains (× 360).

FIGS. 24 and 25. *Vaccinium macrocarpon*. FIG. 24. L.s. floret (× 85). FIG. 25. T.s. floret. Carpel and stamen primordia only (× 85).

FIGS. 26 and 27. *Vaccinium myrtilloides*. FIG. 26. Ovule in megaspore mother cell stage (× 360). FIG. 27. Section of anther wall and sporogenous cells (× 360).

FIGS. 28 and 29. *Vaccinium Oxycoccus*. FIG. 28. L.s. of apical floret primordium. Its meristematic tissue stippled (× 85). FIG. 29. L.s. of basipetally placed floret (× 85).

FIGS. 30 and 31. *Vaccinium Vitis-Idaea*. FIG. 30. L.s. of half floret. Placenta with ovule primordia stippled (× 85). FIG. 31. T.s. portion of anther (× 360).

TABLE I

SUMMARY OF WINTER STAGES

Species arranged in sequence of winter development—the more advanced first

Species	Ovule primordium or nucellus	Female gametophyte or archesporial cells	Integument	Anther	Pollen or sporogenous tissue	Tapetum	Middle wall layers of anther
1. *Epigaea repens*	Nucellus disintegrated	Mature female gametophyte	Fully developed	Mature and dehiscing	Binucleate pollen grains in tetrads	Cell contents disintegrated	Crushed and cell contents going
2. *Chamaedaphne calyculata*	Nucellus disintegrated	Two nucleate gametophyte	Fully developed	Mature	Binucleate pollen grains in tetrads	Cell contents disintegrated	Cell contents disintegrated
3. *Kalmia polifolia*	Nucellus disintegrated	Two nucleate gametophyte	Fully developed	Mature	Uninucleate pollen grains in tetrads	Cell contents disintegrated	Cell contents disintegrated
4. *Rhododendron canadense*	Ovule completely turned	1 to 7 archesporial cells	Enlargement at base of ovule	Mature	Uninucleate pollen grains in tetrads	Cell contents disintegrated	Crushed and cell contents gone
5. *Vaccinium angustifolium*	Ovule completely turned	Mature archesporium	Half way to tip of nucellus	Fully formed	Mature spore mother cells	Differentiated and living	Differentiated and living
6. *Vaccinium myrtilloides*	Ovule completely turned	Mature archesporium	Not differentiated	Fully formed	Mature spore mother cells	Differentiated and living	Differentiated and living
7. *Gaultheria hispidula*	Ovule completely turned	Mature archesporium	Half way to tip of nucellus	Fully formed	Mitosis continuing in sporogenous cells	Differentiated but not sharply	Differentiated and living
8. *Ledum groenlandicum*	Ovule primordium slightly curved	Not differentiated	Not differentiated	Fully formed	Uninucleate pollen grains in tetrads	Cell contents disintegrated	Cell contents disintegrated
9. *Andromeda glaucophylla*	Ovule primodium slightly curved	1 to 2 archesporial cells	Not differentiated	Fully formed	Spore mother cells not yet enlarged	Differentiated and living	Differentiated and living
10. *Arctostaphylos Uva-ursi*	Pendant ovule primordium	Not differentiated	Not differentiated	Anther inverted	Mitosis continuing in sporogenous cells	Differentiation starting	Differentiated and living
11. *Vaccinium Vitis-Idaea*	Ovule primordia barely evident	Not differentiated	Not differentiated	Anther inverted	Sporogenous tissue in some florets	Not differentiated	Two layers undifferentiated
12. *Gaylussacia baccata*	Definite placental primordium	Not differentiated	Not differentiated	Anther inverted	4 groups forming 1 to 4 cells each	Differentiation starting	Two layers undifferentiated
13. *Kalmia angustifolia*	Minute placental primordium only	Not differentiated	Not differentiated	Distal portion of stamen primordium enlarged but no differentiation of reproductive tissues			
14. *Vaccinium macrocarpon*	The pistil primordium is merely a flouted ring of meristematic tissue			Stamen primordia are slightly club-shaped mounds of meristematic tissue			
15. *Gaylussacia dumosa*	Bract, sepal, petal, and stamen primordia differentiated, no sign of carpel primordia						
16. *Vaccinium Oxycoccus*	Bract, sepal, and petal primordia differentiated, no sign of stamen and carpel primordia						
17. *Gaultheria procumbens*	Wholly vegetative bud on rhizome						

The stamen primordia are swollen at the tip indicating the start of anther formation and the four regions of sporogenous tissue may or may not be differentiated, but the number of sporogenous cells seen in cross section varies from none to four or five. The usual number is one or two. The cells next to the sporogenous cells take the stain more deeply, suggesting the start of the tapetum, but they are not sharply differentiated. Outside this embryonic tapetum, there are two or sometimes three layers of living undifferentiated cells (Fig. 31).

The above is for the basipetally placed florets. As in all other racemes in this family, the apically placed florets are in an earlier stage.

Discussion

The evidence presented above will now be reviewed in an attempt to find answers to the questions asked in the introduction. As the facts used for finding an answer for one question must be employed again for answering some other question, a certain amount of repetition is unavoidable.

1. Is the Winter Stage for Each Species Constant or Variable?

Regarding the stage of development in which each of these Ericaceous plants overwinters the evidence presented should be significant for, as stated above, much of the material was collected over a long period. From this material and from the literature there is some evidence that the resting stage may vary slightly. For instance in one collection of *Epigaea repens* the female gametophyte is not mature but is in about the four nucleate stage. Also, from statements made by Palser (12) it is evident that for *Chamaedaphne calyculata*, the condition which appears to be so constant for Nova Scotia is not so constant for other parts of the continent. Finally, the information available regarding *Vaccinium myrtilloides* is based on plants from one clone only so a definite statement cannot be made regarding this species. However, for all other species, regardless of year or locality, the winter resting stage did not vary. Thus the answer to the first question is that each species has a fairly definite resting stage.

2. Is the Winter Resting Stage Related to the Time of Full Bloom?

To aid in answering the question concerning the possible correlation between the winter stage of development and the time of full bloom the species studied are arranged in two parallel columns.

In the column on the left they are arranged according to the stage of winter development, and in the column on the right the arrangement represents the time sequence of full bloom. For the developmental sequence on the left it was fairly easy to arrange all the species except *Ledum groenlandicum*. In this species the pollen grains are nearly mature whereas the ovules are in a very early stage. However, this is the only species for which there could be much doubt regarding its correct position in this list.

In the construction of the second list, however, it was unexpectedly difficult to arrange the species in an approximately correct order. At first it was thought that the necessary information could be obtained by consulting the various herbaria in the province, recording the date of each collection, and then calculating the average for each species. This was done, but the results obtained were without doubt misleading. It was obviously necessary to compare species with species during the same season. So for a number of seasons this was done, and the list given below is the result of the compilation of information from all sources. Even then it is difficult to "date" a racemose inflorescence when comparing it with flowers which are borne singly. For the former may continue to bloom for over four weeks whereas the latter may all come into bloom at once and be withered in 10 days. Thus in making up these lists it is not possible to attain extreme accuracy and a difference in position of one or two from list to list should not be regarded as significant.

The two lists given in Table II indicate that, in most cases, the winter resting stage is related to the time of full bloom. For instance *Epigaea repens* is at the top and *Gaultheria procumbens* is at the bottom of each list, and for most of the other species each species occupies about the same relative position and is in roughly the same order in respect to the other species. However, to have the correlation complete would mean that the predormancy and postdormancy rates of development were similar for all the species. This cannot be true for there are certain marked non-correlations. One example is *Gaultheria hispidula*, which during the winter is in a comparatively early stage, but blooms before *Rhododendron canadense* in which the winter stage is somewhat more advanced. That is, with *Gaultheria hispidula* the postdormancy rate of development must be comparatively faster. Another and much more striking case of the same type is *Arctostaphylos Uva-ursi*. Regarding its winter stage of development, it is No. 10 on the list, but in the sequence-of-bloom list it is No. 3. This species must go through the later stages of development with comparative rapidity. This is substantiated by Fernald's statement (7) that *A. Uva-ursi* "rarely" blooms in October. Certainly the evidence on which Fernald based that statement must have come from plants in which the later development was very rapid. In contrast with these two species with a comparatively more rapid postdormancy development, *Kalmia polifolia* is a species in which the postdormancy development must be comparatively very slow. For owing to its advanced stage of development during the winter, it is No. 3 on that list, but in the sequence-of-bloom list it is No. 9. That is, it blooms after a number of species which during the winter are less advanced. It is evident from the above that the postdormancy rates of development vary from species to species, some being faster and some slower than the average. This makes it impossible to have complete correlation between the two lists, and for the species studied the winter resting stage cannot in all cases be related to the time of full bloom.

TABLE II

TWO LISTS OF THE SPECIES ARRANGED FOR PURPOSE OF COMPARISON

Arranged in sequence of winter development, the more advanced first	Arranged in sequence of full bloom, the early blooming first	Approximate time of full bloom
1. *Epigaea repens*	1. *Epigaea repens*	Mid-April to mid-May
2. *Chamaedaphne calyculata*	2. *Chamaedaphne calyculata*	Late-April to mid-May
3. *Kalmia polifolia*	3. *Arctostaphylos Uva-ursi*	Early-May to late-May
4. *Rhododendron canadense*	4. *Gaultheria hispidula*	Mid-May to late-May
5. *Vaccinium angustifolium*	5. *Rhododendron canadense*	Late-May to early-June
6. *Vaccinium myrtilloides*	6. *Vaccinium angustifolium*	Late-May to mid-June
7. *Gaultheria hispidula*	7. *Vaccinium myrtilloides*	Late-May to mid-June
8. *Ledum groenlandicum*	8. *Andromeda glaucophylla*	Early-June
9. *Andromeda glaucophylla*	9. *Kalmia polifolia*	Early-June to mid-June
10. *Arctostaphylos Uva-ursi*	10. *Ledum groenlandicum*	Early-mid-June to late-June
11. *Vaccinium Vitis-Idaea*	11. *Gaylussacia baccata*	Mid-June to late-June
12. *Gaylussacia baccata*	12. *Vaccinium Vitis-Idaea*	Mid-June to early-July
13. *Kalmia angustifolia*	13. *Vaccinium Oxycoccus*	Late-June to early-July
14. *Vaccinium macrocarpon*	14. *Kalmia angustifolia*	Late-June to mid-July
15. *Gaylussacia dumosa*	15. *Gaylussacia dumosa*	Early-July to late-July
16. *Vaccinium Oxycoccus*	16. *Vaccinium macrocarpon*	Early-July to late-July
17. *Gaultheria procumbens*	17. *Gaultheria procumbens*	Mid-August

3. For Each Stage in the Development of the Embryo Sac or Anther Is There a Definite Accompanying Stage in the Development of the Other?

A very brief study of Table I is all that is necessary to obtain an answer to question No. 3 and the answer is in the negative, but in view of Erickson's work on *Lilium* it is of interest to make for these Ericaceae a closer study of comparative rates of development in the ovule and anther. Erickson as a result of his findings with *Lilium* states (6, p. 739): "such events as meiosis and mitosis of pollen and embryo sac formation, are seen to be precisely integrated with the exponential growth in length of a flower bud as a whole." It is of interest to determine whether or not this principle could be applied from species to species to the members of the Ericaceae included in this study. For this to be possible, the ovule and stamen in any one flower must develop at similar rates and these rates must be more or less uniform from species to species. A slight indication that such is not the case is found in *Epigaea repens* and *Chamaedaphne calyculata*. In both these the pollen grain overwinters in the binucleate stage, but the female gametophyte of *Epigaea* is completely formed, whereas that of *Chamaedaphne* is only in the two nucleate stage. That is, in these two species, although the pollen grains have reached the same stage before winter sets in, the development of the embryo sac in *Chamaedaphne* is comparatively retarded. Greater differences are seen in the ovules of *Kalmia polifolia*, *Rhododendron canadense*, and *Ledum groenlandicum*. In all these the winter stage of the pollen grain is the same, namely uninucleate and in tetrads, but the ovules are in widely different stages as follows: in *Kalmia polifolia* there is a two nucleate female gametophyte and the integument is completely developed; in *Rhododendron canadense*, the archesporial cells are present but the integument is just appearing; in *Ledum groenlandicum*, the ovule is just a slightly curved primordium with no signs of archesporial cells or integument. It is plain that in these three species the embryo sacs exhibit three different rates of development. In the cases cited above, comparatively rapid predormancy development is found in the anther but in *Gaultheria hispidula* the comparatively more rapid development is found in the ovule. In the winter stage of this species, the megaspore mother cell is mature but the sporogenous cells of the anthers are still dividing by mitosis. Comparing this with the winter conditions of *Rhododendron canadense* and *Ledum groenlandicum*, it is evident that the winter ovule of *Gaultheria hispidula* is comparatively advanced but its anthers are in a comparatively early stage. Other cases could be cited but, from the above, it is evident that the ovule and stamen in any one flower do not develop at similar rates. The rates at which they do develop vary greatly from species to species, thus the rates of development of both cannot be referred to one variable such as bud size. Hence, the methods which Erickson found applicable in the case of *Lilium* are not applicable to the forms included in this study. It follows inevitably from this that, for each stage in the development of the embryo sac or anther, there is not a definite accompanying stage in the development of the other.

4. Is There Evidence to Suggest That the Ericaceae Were Evolved in a Region Where It Was Possible for the Plants to Bloom for Most of the Year Without Any Definite Resting Period?

Finally, does this study of winter buds lend support to the opinion of Wulff (13, pp. 146–149), who regards the Ericaceae as being in "disharmony" with the low winter temperatures to which many of this family are now exposed? Perhaps such pure speculation is out of place in a fact-recording article such as this but it gives interest to these otherwise mundane facts. For instance, the winter buds form a continuous series from the purely vegetative bud on the rhizome of *Gaultheria procumbens* to the fully developed flower of *Epigaea repens*. With unusual weather conditions, some member of this group may be found in bloom during any one of the 12 months of the year. This is not a state of affairs one would expect to find in plants originally adapted to a climate with a marked periodicity. Also, during a really dormant state one would not expect to find cell division taking place. Yet active mitosis was observed during the dormant period in representatives of three genera, and in addition this cell division was in progress during the "depth of winter" and on days when the temperature was well below freezing. A third suggestion of disharmony is found in the fact that part of a blueberry plant may be killed by low temperatures which do not kill the average woody plant of our north temperate zone. A suggestion of this was included in a previous article (1), but additional evidence on this subject is now available. In the previous article it was stated that, in the spring, the flower cluster is often terminated by withered tissue, although no such withered tissue was ever found in buds examined during the late autumn or early winter. It was then suggested that this dead tissue might be the dead apical floret or florets. During the past winter (1954–55) an attempt was made to determine the extent of this "winter kill". Buds were collected and examined in the middle of each month. They were taken from plants in an exposed locality where a protective covering of snow was unlikely and actually did not occur. In the collections for October, November, December, and January, all the florets were living. The weather up to that time had been comparatively mild. Then during the end of January and the first of February there was a period of zero weather, and in the collections made just after this "cold snap", 16% of the buds contained dead florets. Again, during the last of February there was another period of zero weather. Then following this the March collection was made, and the proportion of dead florets had risen to 33%. This percentage certainly suggests that these florets had been "frost-killed". Another piece of evidence of a similar type was provided by Mr. L. C. Larose, a member of the staff of the Experimental Station at Normandin, Quebec. He stated (10) that the blueberry growers of Quebec do not have to burn their barrens because, occasionally, there is no snow cover during a portion of the extremely cold winter weather. Under such conditions the blueberry bushes are killed to the ground level. Usually the bushes are well covered by snow, and then they are not killed. Similar statements are made by both Marie-Victorin (11) in

a note following his description of *Vaccinium pennsylvanicum* and by G. N. Fortin (8) in a station bulletin. Also, Brierley and Hildreth (4) who experimented with three species of *Vaccinium*, state ". . . . *V. pennsylvanicum*, apparently cannot endure exposure to such winter temperatures as commonly prevail in the regions to which it is native." Certainly, this sensitivity to subzero temperatures suggests that these plants had not been evolved in a climate which had the extreme cold of our present northern winter. As noted above, the continuous developmental series in the winter stages and the occurrence of cell division during the winter both point to the same conclusion. Thus this study of winter stages does give support to Wulff's theory.

Acknowledgments

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BIOLOGY, CYTOLOGY, AND TAXONOMY OF *COCHLIOBOLUS SATIVUS*[1]

By R. A. Shoemaker[2]

Abstract

Cochliobolus sativus (Ito & Kurib. ex Kurib.) Drechs. ex Dast. produced mature ascocarps on seeds of *Zea mays* L., previously disinfested with mercuric chloride, boiled one minute to kill the embryo and partly immersed in Sach's agar. Isolates of the conidial state, *Helminthosporium sorokinianum* Sacc. ex Sorokin (= *H. sativum* Pamm., King & Bakke), and monoascosporic isolates were hermaphroditic and heterothallic. Uninucleate, spherical spermatia 1.7 μ wide, the sole male agents in plasmogamy, were produced in phialids in slime-filled spermogonia measuring 2.5 × 2.5 mm. Hyphal fusions and conidiation did not initiate the heterokaryon in the ascogonium. Homokaryotic isolates and incompatible crosses produced partially mature ascocarps by differentiation of maternal stroma. Pseudoparaphyses extended downwards from subapical stromatic cells and produced locules. Subsequently in compatible crosses the heterokaryotic ascogenous hyphae grew from the sub-basal pseudoparenchyma and produced a fascicle of asci in the locule. Eight scolecospores, uninucleate at inception, formed after meiosis. The asci had two walls (bitunicate) but the inner wall was not evident in asci filled with spores nor functional in spore discharge. Whole asci extruded from the ascocarp in slime and dehisced in water by a terminal circumscissile rupture. *Cochliobolus* Drechs. was placed in the Pseudosphaeriaceae Höhn. sensu Luttrell in the Pseudosphaeriales Thiessen & Sydow.

Introduction

Cochliobolus Drechs. is a genus of pyrenomycetes that has conidial states in the genus *Helminthosporium* Link. The conidial state of *Cochliobolus sativus* (Ito & Kurib. ex Kurib.) Drechs. ex Dast. is *Helminthosporium sorokinianum* Sacc. ex Sorokin although a later synonym, *H. sativum* Pamm., King & Bakke has been more widely used for this fungus (14). The conidial states of *Cochliobolus* are widely distributed and destructive pathogens of grasses. Yet the perfect states are uncommon in nature and difficult to bring to maturity in the laboratory. Consequently ascocarp morphology has not been used much to resolve taxonomic problems of *Cochliobolus* conidial states. Genetic studies with some species of *Helminthosporium* have likewise been impeded.

The first requisite for the present study was consistent formation of ascocarps of *C. sativus*. Tinline established culture methods to induce ascocarps to form and demonstrated that this fungus is heterothallic (20). The present study was begun using the same compatible isolates of *C. sativus* and methods that Tinline used (20). Despite these precautions ascocarp formation was erratic. Tinline himself found ascocarp formation to be inconsistent (21). The inconsistencies in ascocarp development were resolved when the sexual process in *C. sativus* was discovered.

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Contribution from Cornell University, Ithaca, New York. A portion of a thesis presented to the Faculty of the Graduate School of Cornell University for the Degree of Doctor of Philosophy, June 1955.

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When the biological factors: culture procedure, sexuality, and heterothallism were understood ascocarps were produced regularly. It was then possible to proceed with the cytology of ascocarp ontogeny and ultimately to consider the taxonomic position of *Cochliobolus* based on ascocarp anatomy.

Materials and Methods

Stock Cultures

The isolates of *C. sativus* used in this study are recorded and characterized in Table I. The mating type or compatibility group is designated *A* or *a* after the system used by Tinline (20, 21).

Two albino isolates that lacked brown color in the outer wall of the conidium, conidiophore, etc. were used. The original mutant appeared after treatment of wild-type conidia, designated +, with ultraviolet light (9). Tinline made crosses with this albino isolate and obtained isolates T6 and K3 which were of opposite mating type (21).

The capacity to form ascogonia is designated a female character, ♀, while the formation of spermatia is considered male, ♂. All isolates used were hermaphroditic, ☿.

TABLE I

CHARACTERISTICS OF ISOLATES OF *C. sativus* USED FOR THE PRODUCTION OF ASCOCARPS

Isolate	Mating type	Color	Sex	Source of isolate
18	*a*	+	☿	Tinline (20)
22	*A*	+	☿	Tinline (20)
T6	*a*	Albino	☿	Tinline (21)
K3	*A*	Albino	☿	Tinline (21)
104	*a*	+	☿	Cappellini (3)
104*b*	*A*	+	☿	Cappellini (3)
104*c*	*A*	+	☿	Cappellini (3)
104*d*	*a*	+	☿	Cappellini (3)

Media

The conidial states were grown on a medium that had the carbon–nitrogen ratio adjusted to give abundant conidia without superfluous mycelial growth. The medium consisted of: sucrose, 8.0 gm.; *l*-asparagine, 1.2 gm.; dipotassium hydrogen phosphate, 0.6 gm.; Difco yeast extract, 1.0 gm.; shredded agar, 20 gm.; tap water, 1 liter; and had a pH of 7.0 after autoclaving. The medium is abbreviated S.A.Y.

A mineral medium that would not support appreciable vegetative growth was used in the procedure to produce ascocarps. This medium, Sach's agar (21), was composed of: calcium carbonate, 4.0 gm.; calcium nitrate, 1.0 gm.; dipotassium hydrogen phosphate, 0.25 gm.; magnesium sulphate, 0.25 gm.; ferric chloride, trace; shredded agar, 20 gm.; distilled water, 1 liter. The medium had a pH of 5.3 after autoclaving.

Culture

For the formation of ascocarps the following procedure was utilized. Cereal seeds were disinfested with a solution that contained 10% Clorox and 0.1%

mercuric chloride applied for five minutes. Then the seeds were boiled one minute in sterile distilled water to kill the embryo and rinsed with two changes of sterile distilled water. Six seeds were placed in each 9 cm. Petri dish. Sach's agar was added until the seeds were half immersed. A drop of an aqueous suspension of conidia was added to each kernel. Crosses were routinely made by adding a second drop containing compatible conidia directly afterwards. In special cases crosses were made by transferring spermatia with a brush or transfer loop.

Petri dish cultures were kept uninverted in lightproof, waxed cardboard containers to maintain a high relative humidity during the prolonged (three week) growth period.

Slide cultures were used to observe germination of conidia and the formation of ascogonia. The culture slide was prepared by pouring a thin layer of S.A.Y. on a sterile slide in a sterile Petri dish.

Cytology

Ascocarps were fixed in a Carnoy-type solution (one part propionic acid: three parts absolute alcohol) for meiotic studies. Chromosomes were stained with aceto-ferro-carmine (11). Ascocarp structure was examined in freehand sections and in microtome sections cut at 10 μ of material fixed in F.P.A. (11) and embedded in Tissuemat. Spermogonia were fixed with vapors of osmic acid and formalin, placed in isopropyl alcohol, and embedded. For anatomical studies the nuclei were stained with safranin (11) and the cell walls by means of the periodic-Schiff reaction (8). Phase contrast microscopy was used to accentuate the outlines of cells and chromosomes.

Observations

The results of the investigation with *C. sativus* are recorded in sequence following the development of the ascocarp. The morphology of each reproductive body is treated first. Information on the influence of the culture method on development is then presented. The influences of sexuality and heterothallism are considered finally.

Conidia

Conidia were used in this study chiefly to establish cultures that would form ascocarps. Wild-type conidia were fusoid, with a dark brown outer

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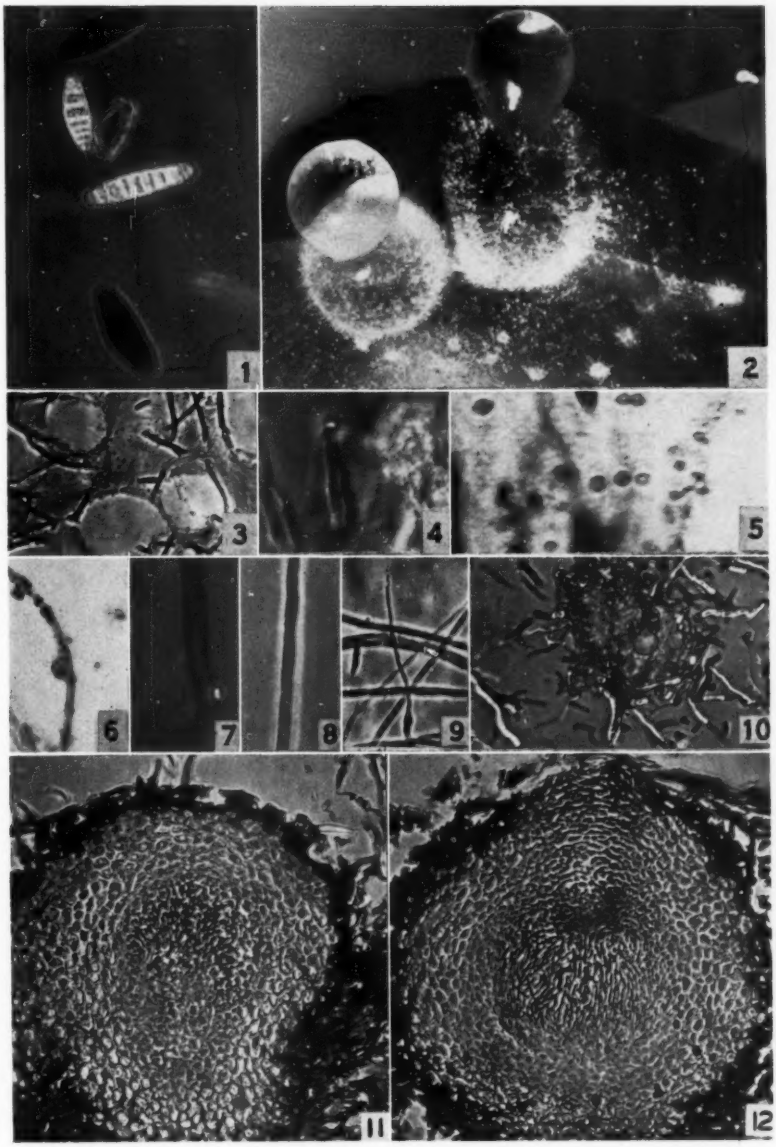
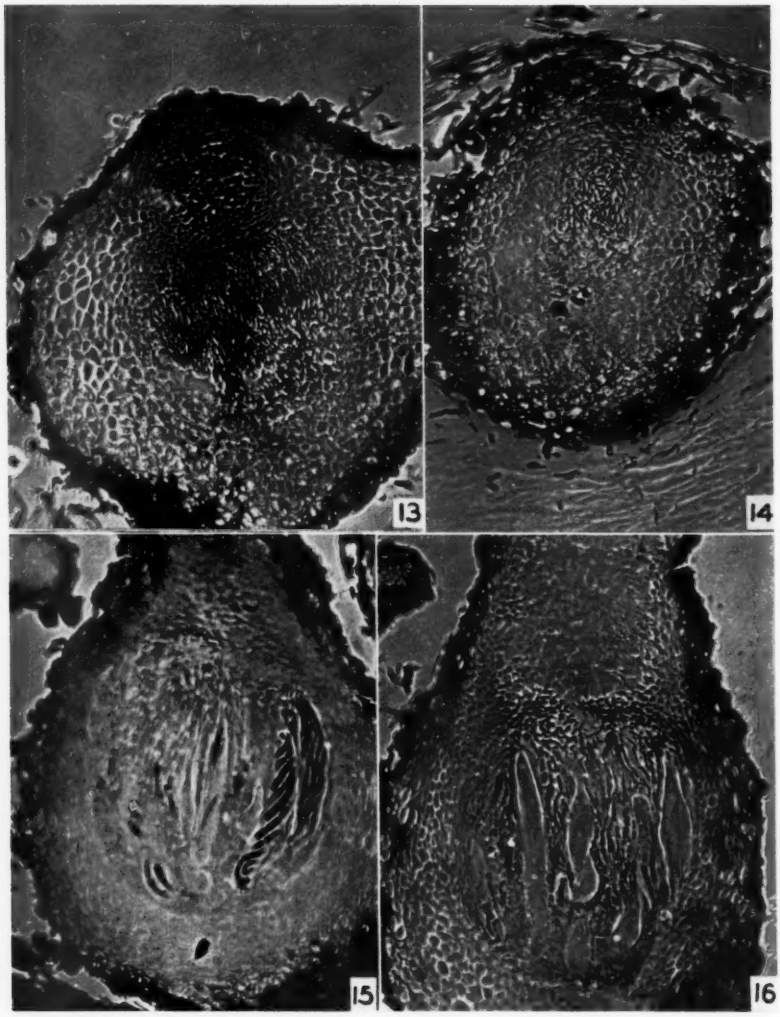
Cochliobolus sativus. FIG. 1. Wild-type conidia from isolate 104*b* and albino conidia from isolate T6. × 200. FIG. 2. Two spermogonia and several ascocarp initials from isolate T6. × 10. FIG. 3. Section of spermogonium of T6 showing vegetative hyphae and spermatia in slime. × 200. FIG. 4. Spermatium on spermatiophore. × 1000. FIG. 5. Spermatia showing nuclei. × 1000. FIG. 6. Nucleate spermatium on trichogyne × 1000. FIG. 7. Enucleate spermatium on trichogyne. × 1000. FIG. 8. Portion of trichogyne showing thin walls, septa, and nuclei. × 1000. FIG. 9. Ascogonium formed in slide culture. × 200. FIG. 10. Ascocarp initial in section showing part of ascogonium. × 200. FIG. 11. Pseudoparaphyses originating from subapical pseudoparenchyma. × 200. FIG. 12. Pseudoparaphyses in the locule and darkly stained cells that may be remnants of the ascogonium in the pseudoparenchyma. × 200.

PLATE I





Cochliobolus sativus. FIG. 13. Developing beak. × 200. FIG. 14. Early stage in development of ascogenous hyphae. × 200. FIG. 15. Basal fascicle of asci. × 200. FIG. 16. Mature pseudothecium with periphyses, pseudoparaphyses, and asci. × 200.

wall and a hydrophylic hyaline inner wall that separated the cells of the mature phragmospore (Fig. 1). Conidia germinated from one cell at each end although all cells were capable of germination if freed from the outer wall.

The optimum temperature for conidial formation was 28° C. and the optimum pH was 7.0. However, conidia formed readily over a wide range of conditions and under all conditions suitable for the formation of ascocarps. Conidia were present on all differentiated reproductive structures formed by *C. sativus*.

Spermogonia

Male reproductive structures of *C. sativus* were unusually large, often 2.5 × 2.5 mm. Fig. 2 illustrates the size and shape of spermogonia relative to the ascocarp initials on the surface of a corn kernel. The exterior of the spermogonium consisted of loosely interwoven hyphae bearing conidiophores with conidia on the outer surface. The spermogonia in Fig. 2 were light colored since they formed from an albino isolate and were covered with hyaline conidiophores and conidia. Some hyaline conidia of this albino isolate are illustrated in Fig. 1. The interior of the spermogonium was filled with slime that exuded in a large drop from the apex (Fig. 2). Vegetative hyphae about 3.2 μ wide, like those of the wall, traversed the central part of the spermogonium (Fig. 3).

Spermatia formed in the central part of spermogonia on spermatiophores scattered through the slime (Fig. 3). The spermatiophores were narrower than vegetative hyphae, about 1.6 μ wide. Spermatia were formed endogenously and extruded singly like phialospores (Fig. 4). Spermatia were mostly 2.0 μ (1.7–2.5 μ) in diameter, more or less spherical and contained a single, central, spherical nucleus usually 1.3 μ (1.0–1.6 μ) in diameter (Fig. 5). The nuclei in the vegetative hyphae in the spermogonia commonly measured 1.6 × 1.3 μ.

Spermatia of *C. sativus* did not germinate when placed on S.A.Y. at 20° C. and left for four days. A few conidia that were transferred with the spermatia from the spermogonium germinated readily; some germ tubes were evident within two hours.

Spermatia were found firmly affixed to trichogynes (Fig. 6). Enucleate spermatia were observed on trichogynes six hours after spermatization (Fig. 7).

The cultural conditions favoring formation of spermogonia have been examined to some extent. Evident spermogonia formed only occasionally on barley seeds but formed regularly on autoclaved corn and corn disinfested with Clorox or with mercuric chloride. Temperature affected the length of time required for spermogonia to form. At 24° C. they formed within three days while at 20° C. cultures prepared in the same way did not have evident spermogonia until the sixth day.

All isolates regularly formed spermogonia under suitable environmental conditions.

Ascogonia

On slide cultures the female reproductive organ, the ascogonium, arose directly from vegetative hyphae. The basal cells were much swollen, usually over 6 μ wide, but gradually tapered to a very narrow apex, or trichogyne, which was about 1.7 μ wide (Fig. 9). The basal cells of ascogonia were plurinucleate prior to spermatization. The trichogyne walls and septa were extremely thin (Fig. 8). The individual cells of the trichogyne had two to six nuclei, usually three, as did the basal cells of the ascogonium. The nuclei in the basal, swollen cells of the ascogonium were spherical and about 1.3 μ wide. Nuclei in the trichogynes were spherical or elliptical with the long axis parallel to the long axis of the trichogyne. Nuclei in the trichogyne measured 1.4 $\times$ 0.8 μ.

On cereal seeds ascogonia appeared first as simple erect outgrowths from vegetative mycelium in or on the pericarp. Branches formed from basal cells of the ascogonium. Each branch was terminated by a uniformly narrow, unbranched trichogyne. Trichogynes often extended one to two millimeters.

In cultures three days to a week old there were usually evident on the surface of seeds, hyaline setose cushions of mycelium (Fig. 2). These were a transitory stage in development termed ascocarp initials. Sections of material at this stage showed the much branched ascogonium made up of inflated, densely cytoplasmic cells (Fig. 10). Around the ascogonium were less densely cytoplasmic cells that were somewhat isodiametric and formed the hyaline stroma. The surface of the stroma was covered by conidiophores and conidia interspersed with trichogynes.

Ascogonia formed within 44 hr. on slide cultures at 20° C. and 24° C. but, unlike the ascogonia on corn seeds, did not develop the surrounding stromatic growth. Ascogonia formed on corn seeds within two days at either 20° C. or 24° C. The development of the ascogonium per se beyond the stage found in the ascocarp initial depended upon successful spermatization by a compatible isolate. Isolates from single ascospores and crosses of isolates of the same mating type did not result in further development of the ascogonium although stromatic portions of the ascocarp initial differentiated considerably and formed the protothecium.

Protothecium

The term protothecium is coined here and will be used in this paper to convey the meaning of an incompletely differentiated ascocarp that superficially resembles the mature ascocarp, be it cleistothecium, perithecium, or, as in the case of *C. sativus*, pseudothecium.

The protothecium of *C. sativus* differed most markedly from the mature ascocarp by the complete absence of a differentiated system of ascogenous hyphae. Protothecia and pseudothecia had a common pattern of development to the point of development of ascogenous hyphae. The protothecium developed from the stromatic ascocarp initial. The first evidence of differentiation was that some subapical cells were densely cytoplasmic (Fig. 11). These cells produced both the pseudoparaphyses and the beak.

Pseudoparaphyses arose as basally directed outgrowths of subapical cells (Figs. 11, 12). In early stages the pseudoparaphyses were free at the lower end and appeared to grow like vegetative hyphae, branching and anastomosing within the locule that formed concomitant with their growth. The tips of the interthecial threads pressed against the sub-basal pseudoparenchyma. The cells composing the pseudoparenchyma in the lower area were not densely cytoplasmic and appeared as though distorted and flattened by the growth of pseudoparaphyses (Fig. 17). However, there was no evident indication that the pseudoparenchyma was digested where it was in contact with the interthecial threads. Pseudoparaphyses recurved where in contact with the sub-basal cells of the stroma so that the free ends of some of the interthecial threads pointed towards the beak (Fig. 17). At the point where the threads recurved a plexus was formed so that some of the interthecial threads were attached at both ends.

The beak of the ascocarp had its origin from the subapical cells of the stroma (Fig. 13). Some of these cells elongated and formed a short beak. The beaks of ascocarps were variable in length and often lacking. Some beaks formed on protothecia but they were more pronounced on mature pseudothecia. The cells of the beak were rectangular in outline in median sections of protothecia. Periphyses arose from the inner cells of the beak and traversed the ostiole (Fig. 16). The ostiole and centrum of protothecia and young pseudothecia were filled with slime. This slime did not appear to originate from dissolved or gelatinized cells of the ascocarp, since no ascocarp cells gave evidence of lysis.

In mixed albino and wild-type cultures only one type of conidium grew on the exterior of a protothecium. Dissections were performed to see if the various tissues of the protothecium were all derived from the maternal parent. An albino protothecium which arose from an ascocarp initial that had been spermatized by an incompatible wild-type isolate was used. Pseudoparaphyses, pseudoparenchyma, and beak tissue were dissected and placed on S.A.Y. and examined for the presence of dark, contaminating conidia. When no wild-type conidia were present the cells were cultured and examined later. Only albino conidia were formed in each instance.

Protothecia formed over a wide range of temperature (9°–24° C.) but only on substrates suitable for the formation of pseudothecia.

Cultures from single ascospores produced only protothecia under conditions that regularly produced ascospore-bearing pseudothecia in compatible crosses. Similarly, crosses between isolates of the same mating type produced only protothecia. There was no fundamental anatomical difference detected between the protothecia formed from single ascospore isolates and those formed in all possible paired combinations of cultures of the same mating type. Isolates 18, 22, T6, K3, 104, and 104*b* produced protothecia regularly while isolates 104*c* and 104*d* produced them only occasionally.

Pseudothecium

The mature ascospore-bearing ascocarp of *C. sativus* is a pseudothecium. According to Nannfeldt (17) this term was coined by von Höhnel. It will be used in this paper to denote the ascocarp formed by members of the Pseudosphaeriales in contradistinction to the perithecium formed by members of the Sphaeriales. The development of the pseudothecium differed fundamentally from that of the protothecium in the formation of ascogenous hyphae. Both structures lacked the continuous ascogonium observed in ascocarp initials (Fig. 10). Instead there were densely cytoplasmic cells, resembling cells of the ascogonium, scattered in the stroma (Fig. 12). The ascogenous hyphae were first evident in ascocarps that had a locule formed by pseudoparaphyses and an initiated beak (Fig. 14). Cells of the ascogonium were connected with the large multinucleate cells that comprised the ascogenous hyphae. The ascogenous hyphal system was relatively short and the asci were restricted to a basal fascicle (Fig. 15). Croziers formed at the apices of ascogenous hyphae. A series of croziers formed in basipetal succession so that the most mature asci appeared at the apex (Figs. 18, 19).

The ascogenous hyphae that originated in the basal pseudoparenchyma extended upwards among the pseudoparaphyses. No case was found where these two systems formed anastomoses with one another. An ascigerous albino pseudothecium formed after a cross with a compatible wild-type was dissected. Groups of cells from the beak, pseudoparenchyma, and pseudoparaphyses all formed only albino (maternal) conidia when cultured.

The procedure used to induce ascocarps to form was modified and improved throughout the study. It was found repeatedly that ascospores were regularly formed only when the culture temperature was 20° C. At 18° C. and 24° C. the formation of ascospores was erratic.

The sort of seed on which ascocarps would mature was examined. Barley was found to be suitable while wheat, oats, and rice supported growth of conidia predominantly. However, corn, *Zea mays* L. 'Robson 320', gave markedly more ascocarps (and spermogonia) than did barley and was adopted for general use.

The addition of vitamins by means of 0.1% yeast extract in Sach's agar gave no stimulatory effect to ascocarp formation. The addition of 0.1% dextrose to Sach's agar resulted in conidial growth on mercury-disinfested seeds instead of the formation of ascocarps.

The methods of sterilizing and disinfesting corn seeds were examined. Seeds that were autoclaved or disinfested with Clorox supported growth of conidia and spermogonia but not of ascocarps. Seeds sterilized with mercuric chloride or a combination of Clorox and mercuric chloride supported the growth of ascocarps without superfluous growth of conidia on the surface of the seeds. Mercuric chloride with Clorox was adopted for routine use. Occasionally there was apparently sufficient residual disinfestant to prevent conidia from germinating on the seeds.

Sexuality

It was conceivable that the sexual structures described above might be nonfunctional, or even if functional, that plasmogamy might occur by other means. Certain distinct possibilities existed for the fusion of compatible cells. One was the fusion of hyphae including receptive hyphae, the trichogynes. It was also possible that conidia functioned as male cells in place of spermatia. These possibilities were tested first in slide cultures, then with cultures on corn seeds.

Isolates of opposite mating type were permitted to grow towards one another on slide cultures of S.A.Y. at 20° C. After 44 hr. the hyphae and trichogynes had intermingled and some conidia had formed. Hyphae formed anastomoses only with hyphae of the same mating type. Trichogynes also fused only with hyphae of the same mating type. No fusions were observed between trichogynes and conidia of either mating type.

Cultures derived from albino conidia were grown for three days on corn seeds until ascogonia were evident. Compatible dark conidia were added along with compatible spermatia. The dark conidia were readily observed on the albino hyphal mat and did not germinate. When some dark conidia were removed from the hyphal mat to S.A.Y. one week later, they grew as promptly as did conidia from cultures on S.A.Y. There was neither growth of dark mycelium observed among the albino hyphae nor fusion between trichogynes and dark conidia. Under these conditions ascocarps still formed ascospores that gave dark or albino cultures within the regular period of three weeks.

Heterothallism

The isolates of *C. sativus* used in this study fell in either group *A* or *a* (Table I) depending upon their ability to form ascospores when crossed. All isolates were self sterile, intragroup sterile, and intergroup fertile.

An additional factor distinct from heterothallism was observed in two isolates, 104*c* and 104*d*. These isolates produced protothecia irregularly and could be classified for mating type only on the presence or absence of asci, since ascospores did not mature. Crosses between 104*c* or 104*d* and compatible albino isolates resulted in the formation of albino pseudothecia that contained asci. No dark protothecia or pseudothecia formed in crosses of this sort. Isolates 104*c* and 104*d* were the least fertile of any studied. Isolates 18, 104*b*, 104, and T6 regularly formed numerous asci and ascospores in crosses while the other isolates produced asci and ascospores sparingly. Only the highly fertile isolates were used in the cytological study.

Ascus Cytology

Croziers formed at the ends of ascogenous hyphae in the pseudothecia. In some croziers two nuclei were evident (Fig. 19). In other croziers two nuclei were found simultaneously undergoing mitosis as in the conjugate division (Fig. 18). Two of the four daughter nuclei were included in the penultimate cell. In penultimate cells that had enlarged somewhat as the initial stage in

development of the ascus there were two nuclei each with one nucleolus (Fig. 19). In asci that were slightly larger the fusion nucleus had only one large nucleolus (Fig. 20) that varied from 3.0 μ in diameter at early pachytene to 6.5 μ at late pachytene stages in meiosis.

Synapsis occurred prior to any appreciable elongation of the ascus (Fig. 21). The synapsed chromosomes were very short and densely stained in this early pachytene stage. The longest chromosome in a typical small ascus, 40 × 16 μ, was 4 μ long. Homologous chromosomes were still synapsed in asci that had reached nearly mature length. However the chromosomes were much longer in this late pachytene stage. The longest chromosome in a representative large ascus, 92 × 21 μ, was 12 μ long. In the larger asci the chromomere pattern was evident on the extended paired chromosomes. They appeared as single threads, synapsed chromomere for chromomere (Figs. 22, 23).

Eight uninucleate spores were delimited in the asci formed following crosses of the more fertile isolates. Spores that had not elongated fully had a nucleus near each end (Fig. 24). Spores that had reached their maximum length were coiled in a tight helix within the ascus (Fig. 28). The mature ascospores were multicellular and each cell contained from 6 to 10 nuclei.

In several crosses less than eight ascospores were produced. Occasionally aberrant spherical spores were formed (Fig. 25).

The wall of the ascus is at first thin (Figs. 19, 20). Asci with only partially mature spores had a thick inner wall, the endotunica, and a thin rigid ectotunica. In young asci the endotunica was penetrated by an apical canal filled with cytoplasm (Fig. 26). The endotunica was made to extrude by breaking the ectotunica with gentle pressure on the cover glass of water mounts of immature asci (Fig. 27). A similar differentiation into a rigid outer wall and an amorphous hydrophilic inner wall exists in the conidia (Fig. 1).

The endotunica was not visible in asci that were filled with coiled scolecospores (Fig. 28). Mature asci were extruded in slime and collected on the beak of the pseudothecium in culture. The base of the ascus showed no sign of lysis and the pores in the basal septa were plugged (Fig. 29).

Since whole asci rather than spores were discharged from the ascocarp, asci were mounted in hanging drops of distilled water to follow ascus dehiscence. Some asci broke open almost immediately and the spores uncoiled and extended from the ascus. Asci commonly opened by a circumscissile apical

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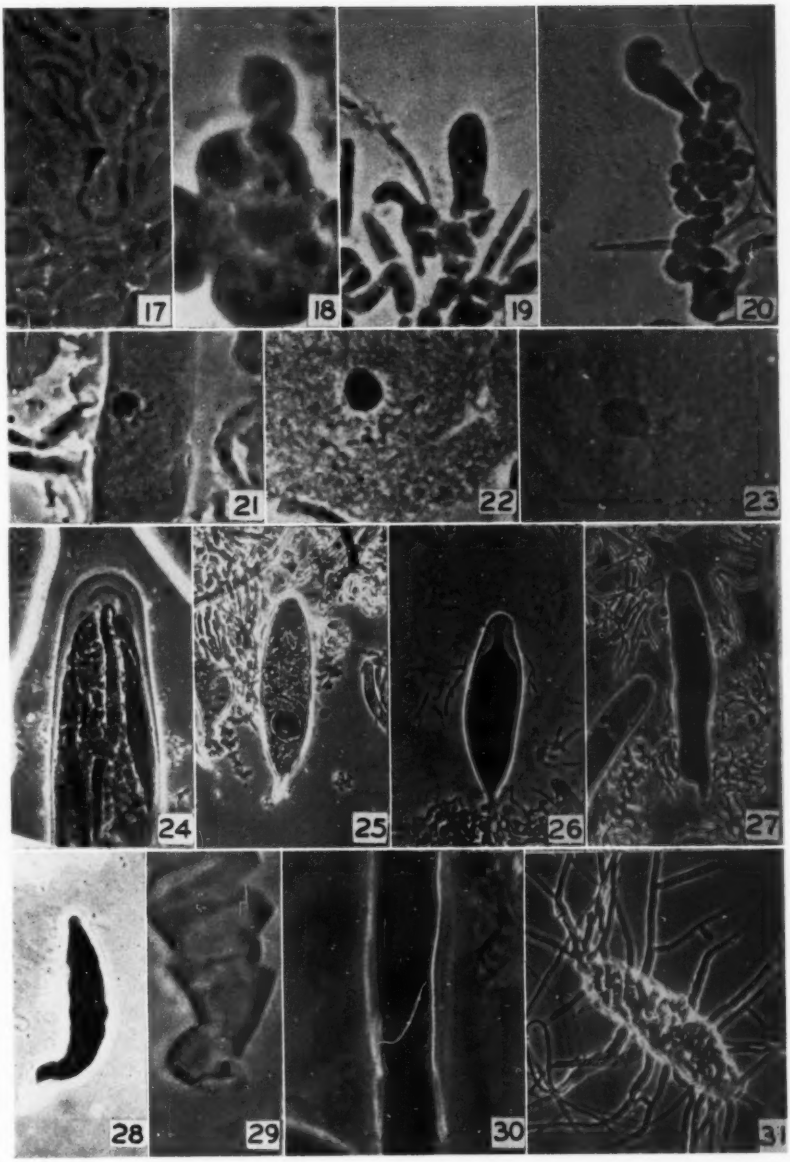
Cochliobolus sativus. FIG. 17. Recurved pseudoparaphysis. × 1000. FIG. 18. Anaphase of conjugate division. × 2000. FIG. 19. Repetition of croziers and young ascus with two nuclei each with one nucleolus. × 1000. FIG. 20. Ascus with fusion nucleus and repetition of croziers. × 500. FIG. 21. Pachytene showing chromosomes that have elongated slightly from the earliest pachytene stage. × 1000. FIG. 22. Later pachytene. × 1000. FIG. 23. Pachytene illustrating maximum elongation of synapsed chromosomes. × 1000. FIG. 24. Young binucleate ascospores at the tip of an ascus. × 500. FIG. 25. Aberrant spherical spore. × 200. FIG. 26. Ascus prior to spore formation showing an apical canal in the endotunica. × 200. FIG. 27. Extruded endotunica containing some of the cytoplasm that filled the apical canal. × 200. FIG. 28. Thin-walled mature ascus. × 200. FIG. 29. Plugged pores in the base of an ascus. × 1000. FIG. 30. Hydrophilic sheath on fragmented ascospore. × 1000. FIG. 31. Ascospore germination in an ascus. × 200.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rupture but also broke open at the center and base occasionally. The spores from mature asci developed a gelatinous hydrophilic sheath when they were released in water (Fig. 30). No evidence of an endotunica functioning in spore discharge could be obtained at this stage because of the sheath on the spores. In asci that had released mature spores there was no evident residual endotunica.

Some asci did not rupture to release spores. Instead the ascospores germinated *in situ* and hyphae grew directly through the ascus wall (Fig. 31).

Discussion

Morphological Aspects in Ascocarp Formation

The spermogonia and ascogonia of *C. sativus* have apparently been overlooked by previous workers. Spermogonia are bizarre structures, much larger than the pseudothecia of *C. sativus*. Had they been formed in cultures used by other workers they would assuredly have excited some comment. Drechsler found and figured somewhat similar structures in *Ophiobolus* sp. (7). Microconidia figured by Drechsler were produced on narrow annellate conidiophores within thin-walled pycnidia. But the spermogonia of *C. sativus* were much larger than the pycnidia of *Ophiobolus* sp. and the spermatiophores of *C. sativus* were phialids, not annellate conidiophores. The size of the spermogonia of *C. sativus* depends largely upon the amount of slime present and scarcely constitutes a firm distinction from the pycnidium of *Ophiobolus* sp. However, the mode of formation of *Ophiobolus* microconidia is entirely different from the formation of spermatia on phialids and shows the distinctness of these two structures.

Backus drew attention to the distinctions between spermatia and microconidia (1). The male reproductive cells in *C. sativus* are spermatia since they are agents of plasmogamy and fail to germinate under conditions suitable for the germination of conidia.

The ascogonium of *C. sativus* is a much-branched, elongate hyphal system in the ascocarp initial. However, in prototheeia and pseudothecia the ascogonium could not be discerned as a continuous structure. The densely cytoplasmic cells scattered in the stroma round the locule may represent fragments of the ascogonium. In any case the functional portion of the ascogonium appears to reside near the base of the pseudothecium since it is from this position that the ascogenous hyphae originate. Dissection experiments indicated that the stromatic tissue around the locule was homokaryotic and maternal in origin. If the densely cytoplasmic cells scattered in the stroma were ascogonial remnants they did not give evidence of being heterokaryotic. The cells of the ascogonium in the basal pseudoparenchyma that give rise to ascogenous hyphae are heterokaryotic since the asci derived from them contain asci with spores segregating for paternal and maternal characteristics.

Prototheeia have been reported in most species of *Cochliobolus* (10, 15, 20) and in *Helminthosporium turcicum* Passerini (10) which might be expected to

give a *Cochliobolus* stage. The development of the protothecium involves stromatic differentiation. The pseudoparaphyses extend and form a locule in the stroma. The subapical cells initiate a beak. Development is then arrested. The protothecium is predominantly and, as far as these experiments show, exclusively maternal and homokaryotic since it develops in monoascosporic cultures and dissections of protothecia formed after incompatible crosses gave no indication of heterokaryotic nature. Furthermore, the heterokaryotic ascogenous system does not develop as in pseudothecia.

The pseudothecium is distinguished from the protothecium of *C. sativus* by the development of ascogenous hyphae. These hyphae are distinct from other tissues in time and place of origin and in genetic constitution. They are the last hyphal system formed in the centrum and arise in the sub-basal pseudoparenchyma whereas the pseudoparaphyses and beak originate from subapical pseudoparenchyma. The ascogenous hyphae are heterokaryotic while the other tissues of the ascocarp are homokaryotic.

Meiosis in *C. sativus* departs somewhat from the classical stages in the meiotic prophase. However it does not differ fundamentally from the description given by Singleton for *Neurospora* (19). The presynaptic stage that corresponds in timing to leptotene is unusual since the chromosomes are short and densely staining. The interval between the conjugate division and synapsis is relatively short on the basis of the infrequency of asci showing presynaptic stages. Apparently synapsis occurs shortly after the conjugate division and prior to any appreciable elongation of the chromosomes in the fusion nucleus.

Synapsis occurs between short, densely staining chromosomes. The paired chromosomes then elongate until they are about three times as long as they are when synapsis occurs. In this stage of maximum elongation (late pachytene) the pairing of the homologues gave no evidence of structural hybridity.

Some of the crosses that produced few ascospores and many aborted asci conceivably had meiotic irregularities. These are not unexpected since the sexual stage occurs so rarely in nature that it has not been reported and the fungus reproduces asexually over a wide area.

Tinline found the ascospores of *C. sativus* to be uninucleate at inception (21). His genetic studies substantiated this observation (20, 21). The present work confirms this result. Thus, although the spores are multicellular and each cell is plurinucleate, each ascospore is homokaryotic.

Taxonomic Position of Cochliobolus

Recent studies of pyrenomycete taxonomy are based largely on ascocarp anatomy. The systems of Nannfeldt (17), Miller (16), and Luttrell (13) were consulted in the present study. These workers make the primary division of pyrenomycetes on ascohymenial vs. ascolocular, perithecial vs. pseudothecial, and unitunicate vs. bitunicate structures, respectively. These views represent differences in emphasis and are mutually complementary in most respects that affect *Cochliobolus*.

In the system used by Nannfeldt, *C. sativus* falls in the ascolocular group since the asci arise in a basal fascicle rather than in a widespread hymenium.

The fundamental distinction between perithecial and pseudothecial forms is made in Miller's treatment on the presence in perithecial forms of a wall that surrounds the young ascogonium and separates it and the developing centrum from the stroma. The pseudothecial forms on the other hand have a centrum that develops as a locule in stroma. The centrum of *C. sativus* develops in a loculate stroma without a surrounding wall distinct from the isodiametric cells of the stroma. The locule forms through extension of pseudoparaphyses, and asci subsequently develop in the locule. In mature ascocarps the stroma around the single locule simulates a wall but does not constitute a perithecial wall in the sense of Miller.

Luttrell emphasized the nature of the ascus to separate perithecial and pseudothecial forms. The former, unitunicate, group has an ascus thickened only at the apex where it is penetrated by a pore and is often supplied with a refractive ring. The members of the bitunicate group have a uniformly thin, rigid, outer wall and, during development, an additional thick, extensible, hydrophilic inner wall. *C. sativus* has a bitunicate ascus. It differs from the commoner bitunicate forms like *Pleospora herbarum* in that the endotunica does not appear to function in discharge of spores from the pseudothecium. Rather, whole asci are extruded in slime and collect on the beak of the ascocarp.

The pseudothecial pyrenomycetes that have pseudoparaphyses fall in Pseudosphaeriales Thiessen & Sydow sensu Miller. Luttrell (13) suggested that this order might be called Pleosporales since *Pseudosphaeria* was transferred to the Myriangiales. But in the system of classification Luttrell presented (13), *C. sativus* falls in the Pseudosphaeriaceae Höhn. together with *Pyrenophora*, *Leptosphaeria*, *Pleospora*, and others.

From the evidence presented by Drechsler (6) it is known that *C. heterostrophus* (Drechs.) Drechs. has pseudoparaphyses and ascoloculate ascocarps. Hence it would appear that *C. heterostrophus* belongs in the Pseudosphaeriaceae along with *C. sativus*. Since *C. heterostrophus* is the type of *Cochliobolus* the genus is placed in the Pseudosphaeriaceae.

Extrinsic Factors in Ascocarp Formation

Temperature has a pronounced influence on development of spermogonia. A temperature of 24° C. brings spermogonia to maturity when ascogonia are first formed. Lower temperature delays the formation of spermogonia. It would therefore seem advisable to use an initial culture temperature of 24° C. to facilitate spermatization, for about one week, the length of time spermogonia are usually present in cultures.

The development of ascogonia and prototheca is not much affected by small differences in incubation temperature. However, ascosporogenesis occurs regularly only at 20° C. Tinline also found this to be true (21). *C. stenospilus* T. Mats. & Yamamoto *nom. nud.* also has a precise optimum

for ascosporogenesis, but it is at 25° C. (15). After the initial period at 24° C. *C. sativus* should be cultured at 20° C. to permit ascospores to mature. This is the procedure that Tinline adopted although no explanation was offered for the seemingly empirical method (21).

Tinline found that light inhibited ascospore formation in ascocarps (21). All cultures used in the present study were grown in lightproof containers, so no differential effect of light and darkness was observed.

Corn seed is the most suitable substrate found for the formation of spermogonia and pseudothecia. It is markedly better than barley and other seeds used by Tinline (20). The spermogonia of *C. sativus* may have been overlooked by other workers who used substrates that did not regularly support the growth of readily visible spermogonia.

The method of freeing corn from contaminating microorganisms affects ascocarp production. Autoclaved seeds supported growth of conidia and spermogonia predominantly. Autoclaving is known to hydrolyze starch (12) and probably affects the fungus by means of increased sugar concentration, since dextrose added to mercury-disinfested seeds favored conidial formation. No such mechanism can be postulated for Clorox-disinfested seeds, on which only spermogonia and conidia were evident. Mercuric chloride added to Clorox or alone is the disinfestant that best conditions seeds for growth of pseudothecia, even though germination of conidia on the surface of seeds is sometimes suppressed, presumably as a result of residual mercury. Mercury affects sulphydryl-containing enzymes responsible for carbohydrate metabolism (12). With *C. sativus* the effect of mercury was reversed by added dextrose. Mercuric chloride may act through carbohydrate metabolism and thereby influence ascocarp formation. The mercury might also act to suppress surface growth of conidiophores and leave unaffected the mycelium in the cells of the pericarp, from which the ascocarp arises. In any case those who wish to duplicate these results must consider the function of mercuric chloride to be more complex than that of a surface disinfestant.

Sexuality

Genetic studies with *C. sativus* indicated that it undergoes plasmogamy, karyogamy, and meiosis like other sexually reproducing pyrenomycetes (20, 21). However, the descriptions of spermogonia and ascogonia given above are believed to represent the first record of male and female structures in *Cochliobolus*. Spermatial nuclei penetrate the trichogynes and the basal cells of the ascogonium eventually become heterokaryotic as evidenced by the asci and ascospores produced from them. Conidia apparently do not function as agents of plasmogamy since they were not found fused with the receptive hyphae as they are in *Neurospora* where conidia function as male elements (2). Also unlike the condition in *Neurospora* (5), there was no fusion observed between trichogynes and compatible hyphae. In this connection Tinline found hyphal fusions between isolates of opposite mating types to be extremely rare and was unable to demonstrate that a heterokaryon had been formed after this fusion (21). It seems reasonable to conclude that spermatia are the

sole spermatizing agents in *C. sativus*. Some inconsistencies in the biology of *C. sativus* might be resolved if the problems were re-examined with the knowledge of the function of spermatia. Evident spermogonia formed erratically on media similar to those used by others, but it is probable that spermatization was effected by spermatia formed either in minute spermogonia or outside an organized structure.

Heterothallism

Tinline found that monoconidial isolates of *C. sativus* from different geographic regions fell into the two compatibility groups in approximately equal proportions (20). This was confirmed in the present study. The genetic basis of heterothallism is attributed to an allelic gene pair in *C. sativus* (20, 21). No cytological evidence, such as a heteromorphic pair of chromosomes, was obtained that would disagree with this conclusion. For the present it may be concluded that *C. sativus* exhibits bipolar heterothallism controlled by an allelic gene pair.

Other species of *Cochliobolus* may not be heterothallic. Dastur found mature ascocarps of *C. tritici* Dast. formed from a monoconidial isolate on one occasion (4). Nisikado found that ascocarps matured in a culture derived from a single conidium of *C. kusanoi* (Nisikado) Drechs. ex Dast. (18). Contamination of cultures or presence of heterokaryotic conidia are possible alternatives to the conclusion that there are homothallic species in *Cochliobolus*. This aspect requires re-examination with cultures known to be derived from homokaryotic cells.

Heterothallism in *C. sativus* appears to act by arresting ascocarp development at the protothecium stage. Prototheca from monoascosporic cultures or incompatible crosses have fairly complete differentiation of the tissues of stromatic origin but lack differentiation of the heterokaryotic ascogenous hyphae.

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THE SPECIES OF *CIBORINIA* ON *POPULUS*[1]

By J. Walton Groves and Constance A. Bowerman[2]

Abstract

The controversial nomenclatural problem concerning the fungi causing the ink spot disease of poplar is discussed in detail. It is concluded that the correct name of the eastern species is *Ciborinia whetzelii* (Seaver) Seaver and that the western species does not possess a legitimate name. *Ciborinia seaveri* nom. nov. is proposed for this fungus. Two other species of *Ciborinia* on *Populus* are described from specimens and manuscript notes of the late Professor H. H. Whetzel, *C. pseudobifrons* Whet. sp. nov. and *C. davidsoniana* Whet. sp. nov.

The genus *Ciborinia* was established by Whetzel (1945 (10)) and included six species. Two of the species occur on leaves of *Populus* and these have been the source of some taxonomic and considerable nomenclatural confusion. The first object of this account is to establish the correct names for these two species which Whetzel called *Ciborinia bifrons* (Whet.) Whet. and *C. confundens* (Whet.) Whet. *C. bifrons* (Whet.) Whet. was made the type of the genus *Ciborinia*.

Besides these two species, Whetzel also treated three others occurring on *Populus* in his manuscript notes which have been generously placed at our disposal by Dr. R. P. Korf, Cornell University. One of these, which he called *Ciborinia pseudobifrons*, has been collected regularly in the Ottawa district since 1936 where it occurs in abundance every spring. We have carried out some experiments on its sexual behavior and consider that it should be formally named and described. The description of the fourth species will also be published at this time to complete a treatment of the species of *Ciborinia* on *Populus*. For reasons given later, we consider that his fifth species is not a distinct species but is simply *Ciboria acerina* Whet. & Buchw. occurring on leaves of *Populus*.

A consideration of the nomenclature of the ciborinias on poplar can best begin with the sclerotial state. This state occurs on living leaves of *Populus*, forming black sclerotia that create the impression of a blot of ink on the leaves and have prompted the name ink spot disease for the abnormality. The sclerotia eventually become detached and fall to the ground.

Specimens consisting of leaves bearing these sclerotia were distributed in Ellis' N. Amer. Fungi 2554 under the name *Sclerotium bifrons* Ell. & Ev. but with no diagnosis. This is, therefore, a *nomen nudum*.

Saccardo and Sydow (1899 (3)) provided a diagnosis under the name *Sclerotium bifrons* Ell. & Ev. and cited Ellis' N. Amer. Fungi 2554. The valid publication of *Sclerotium bifrons*, therefore, dates from 1899 and it should be

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cited *S. bifrons* Ell. & Ev. ex Sacc. & Syd. (Art. 58 of the International Code of Botanical Nomenclature as amended at Stockholm, 1950). Unquestionably the holotype is the specimen No. 2554 distributed in Ellis' N. Amer. Fungi which, according to the label, was collected at London, Ontario, by John Dearness but, from notes and correspondence with Dr. Dearness found among the papers of the late Professor Whetzel, this specimen was actually collected by Mrs. John Dearness in northern Muskoka, Ontario, and sent to Ellis by Dr. Dearness from London.

Sclerotium bifrons Ell. & Ev. ex Sacc. & Syd. is the correct name for the sclerotial state of the fungus, but must yield precedence to the name of the perfect state (Art. 69). Apparently the first published reference to what was thought to be the perfect state of *Sclerotium bifrons* was made by Seaver and Shope (1930 (5)). In a semipopular account of a collecting trip in the mountains of Colorado they related finding a sclerotial fungus in the leaves of aspen poplar. They thought that this was *Sclerotium bifrons* but, since it has later been shown that the western fungus is a different species from the eastern, and the type of *S. bifrons* is the eastern species, this was a misidentification on their part. They found apothecia arising from sclerotia on the ground and concluded erroneously that they had found the perfect state of *Sclerotium bifrons*.

They did not write a formal technical description nor cite any particular specimen of their fungus but did write a few descriptive sentences in narrative style that can be accepted as meeting the minimum requirements for valid publication. They introduced the binomial *Sclerotinia bifrons* in the following sentence, "Since this discovery was made, Professor H. H. Whetzel of Cornell University, who has made a careful study of this genus, reports having seen apothecia of the fungus *Sclerotinia bifrons* near Ithaca but, so far as can be discovered, these observations have not yet been published." Clearly the binomial, as used here, refers to Whetzel's material collected near Ithaca. This is the only place where the name *Sclerotinia bifrons* is used in the text of the article, but it was also used as a caption for a plate illustrating the western fungus and it is evident from the context that they intended the name to apply to the western fungus. Thus, the position in 1930 was that *Sclerotinia bifrons* Seaver & Shope was a validly published name applying to both eastern and western fungi which were thought to be a single species, but for which no type was designated.

Hubert (1931 (2)) referred to the ink spot of aspen as being caused by *Sclerotinia bifrons* Ell. & Ev. He stated that "Seaver found the ascogenous stage of the fungus on overwintered infected leaves in Colorado and identified it as *Sclerotinia bifrons* E. & E." This statement is incorrect for Ellis and Everhart never proposed the combination and Seaver and Shope did not ascribe it to them.

Boyce (1938 (1)) with reference to the same disease stated "It is caused by *Sclerotinia bifrons* (E. & E.) Seaver & Shope (Ascomycetes, Helotiaceae) formerly known as *Sclerotium bifrons*." This citation is also incorrect for

Seaver and Shope did not propose a new combination based on the Ellis and Everhart name and, if they had, it would be a direct contravention of Article 69 which states "The author who first describes a perfect state may adopt the specific epithet of the imperfect state, but his binomial for the perfect state is to be attributed to him alone and is not to be regarded as a transfer."

Although these improper citations by Hubert and Boyce were confusing they did not affect the legal status of the name *S. bifrons* Seaver & Shope.

Whetzel (1940 (9)) presented conclusive evidence that the eastern and western collections were distinct species. He did not accept *Sclerotinia bifrons* Seaver & Shope as the name for the western fungus although he said that a strict interpretation of the rules would necessitate this action. He proposed that since the name *Sclerotinia bifrons* Seaver & Shope "involved an error in identification" and the specific epithet "is universally associated with the common sclerotial fungus on *Populus*", the eastern fungus should be *Sclerotinia bifrons* Whet. n. sp. At the same time Whetzel formally described the western species as *S. confundens* Whet. n. sp. He provided Latin diagnoses and designated type specimens for both species.

However, Article 63 of the Code states "When a species is divided into two or more species, the specific epithet must be retained for one of them, or (if it has not been retained) must be reinstated. When a particular specimen was originally designated as the type, the specific epithet must be retained for the species including that specimen. When no type was designated, a type must be chosen."

Whetzel did not retain the specific epithet of Seaver & Shope so that this must be reinstated for one of the taxa. No particular specimen was designated by Seaver and Shope as the type, hence the types designated by Whetzel for these taxa must be accepted. *Sclerotinia bifrons* Seaver & Shope could, under these circumstances, be accepted as the name for either of these taxa. It is clear from the later papers of both Whetzel and Seaver that they both considered the Seaver and Shope name to apply primarily to the western fungus. It would seem not only logical, but required by the Code as indicating the intent of the authors, to accept the interpretation that *Sclerotinia bifrons* Seaver & Shope is the name of the western fungus. The nomenclatural consequences of this decision are as follows.

The name *S. bifrons* Whetz. is an illegitimate later homonym of *S. bifrons* Seaver & Shope, and *S. confundens* Whet. is illegitimate because it is a synonym of *S. bifrons* Seaver & Shope and was nomenclaturally superfluous when proposed (Art. 73).

Thus the eastern species was left without a legitimate name and Seaver (1940 (6)) proposed *Sclerotinia whetzelii* nom. nov. for it.

When Whetzel (1945 (10)) established the genus *Ciborinia*, he designated the eastern fungus as the type species under the combination *Ciborinia bifrons* (Whetz.) Whetz. based on the illegitimate *S. bifrons* Whetz. instead of the

legitimate *S. whetzelii* Seaver. Seaver (1951 (8)) reinstated the legitimate epithet in the combination *Ciborinia whetzelii* (Seaver) Seaver for the eastern fungus.

When Whetzel (1945 (10)) transferred the western fungus to *Ciborinia*, he used the combination *C. confundens* (Whet.) Whet. and included *S. bifrons* Seaver & Shope in synonymy although it is the prior and legitimate name. Seaver (1951 (8)) attempted to correct this by a new combination *Ciborinia bifrons* (Ell. & Ev.) Seaver. However, this name is an illegitimate later homonym of *C. bifrons* (Whet.) Whet. and furthermore it is an incorrect citation because, as noted above, it is not permitted under the Code to make a new combination for a perfect state based on the specific epithet of the imperfect state (Art. 69). The epithet *confundens* cannot be taken up legitimately (Art. 81); hence the western fungus is without a legitimate name, and the name **Ciborinia seaveri** nom. nov. is proposed for it.

The specific epithet *bifrons* for the perfect states of the ink spot fungi on poplar would then disappear and the complete synonymy of these two species is as follows:

Ciborinia whetzelii (Seaver) Seaver North American Cup Fungi (Inoperculates), p. 70. 1951. (Figs. 1, 2, 8*a*).
Sclerotinia whetzelii Seaver. Mycologia, 32 : 127. 1940.
Sclerotinia bifrons Whet. Mycologia, 32 : 126. 1940 (non *S. bifrons* Seaver & Shope. Mycologia, 22 : 3. 1930).
Ciborinia bifrons Whet. Mycologia, 37 : 668. 1945 (non *C. bifrons* Seav. N. Amer. Cup Fungi (Inoperculates), p. 71. 1951).

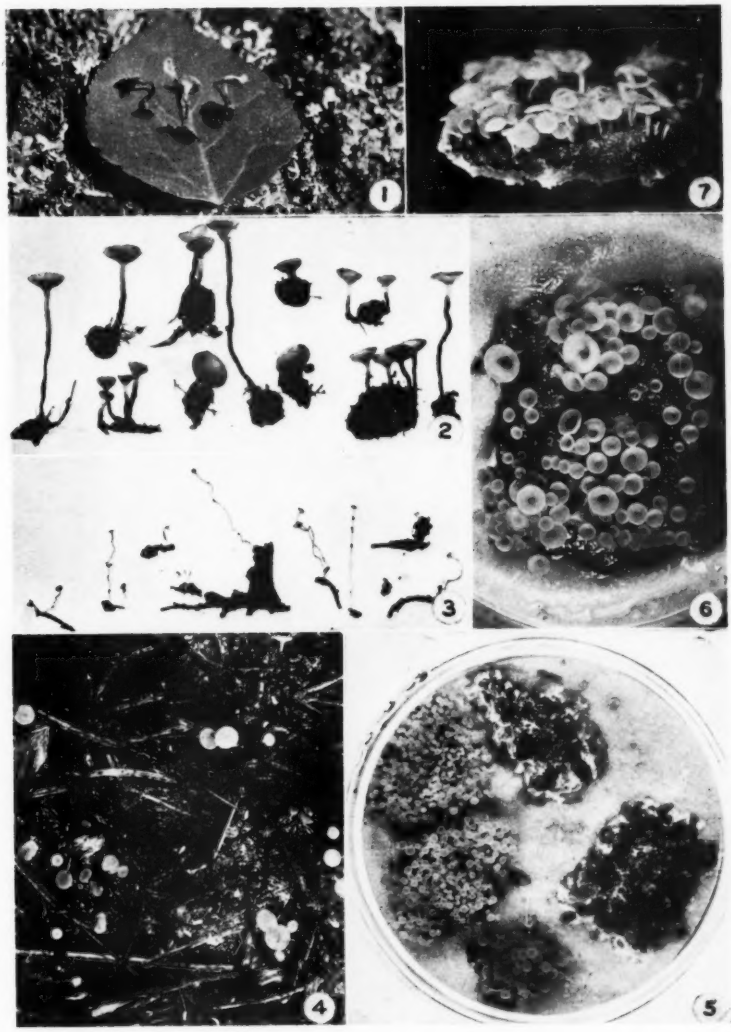
St. sclerot.
Sclerotium bifrons Ell. & Ev. nom. nud. Ell. N. Amer. Fungi, 2554. 1890.
Sclerotium bifrons Sacc. & Syd. Syll. Fung. 14 : 1169. 1899.

Type: CUP 15624.

Type Locality: McLean, N.Y.

Specimens examined: Type CUP 15624. McLean, N.Y. May 23, 1927, Tenaga, Que. DAOM 4594, 5489, 5491, 12044, 26260. Merivale, near Ottawa, Ont. DAOM 23342, 23343, 37534, 37535, 37543, 37556, 37569, 39401. McLean, N.Y. DAOM 4462, CUP 25276.

The earliest and latest dates of collection of the specimens cited above are May 1 and May 26. Apothecia discharge spores freely and sometimes a short germ tube is produced on agar but no further growth takes place. The asci in these specimens measured (100)140–180(200) × (7.5)9–13 μ and the spores (9)11–15(17) × (5)6–8 μ (Fig. 8*a*). The length of the asci varies considerably in different apothecia. Usually they are 140–180 μ long but in some apothecia they are mostly over 170 μ and in one specimen none were observed over 140 μ in length. A striking field character of the species is the presence of rhizoids at the base of the stipe where it arises from the sclerotium (Fig. 2).



FIGS. 1–2. Apothecia of *Ciborinia whetzelii* collected in nature. FIG. 3. Apothecia of *C. pseudobifrons* from nature at same magnification as Fig. 2. FIG. 4. Apothecia of *C. pseudobifrons* in nature among forest debris. FIG. 5. Dish containing one mass and four single ascospore isolates of *C. pseudobifrons* all spermatized with spermatia from the same single ascospore isolate illustrating fertile and infertile crosses M = 0.6. FIGS. 6–7. Apothecia of *C. pseudobifrons* produced in culture.

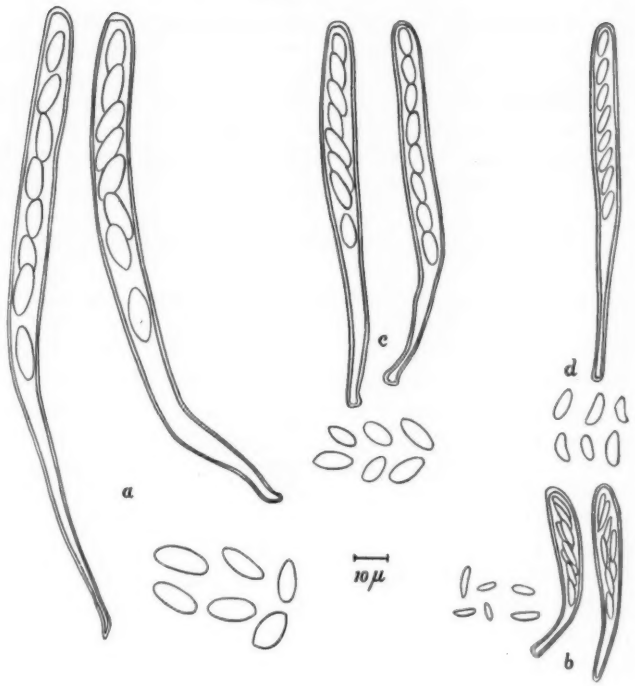


FIG. 8. Drawings of asci and ascospores all made from the types.

(*a*) *Ciborinia whetzelii* (*c*) *C. pseudobifrons*
(*b*) *C. seaveri* (*d*) *C. davidsoniana*

Ciborinia seaveri nom. nov. (Fig. 8*b*).

Sclerotinia bifrons Seaver & Shope. Mycologia 22 : 3. 1930 (non *S. bifrons* Whetz. Mycologia, 32 : 126. 1940).

Ciborinia bifrons Seaver. North Amer. Cup Fungi (Inoperculates), p. 71. 1951 (non *C. bifrons* Whet. Mycologia, 37 : 668. 1945).

Sclerotinia confundens Whet. Mycologia, 32 : 126. 1940.

Ciborinia confundens Whet. Mycologia, 37 : 668. 1945.

Type: CUP 17867.

Type Locality: University of Colorado Camp, Colo.

Through the kindness of Dr. R. P. Korf, the type specimens of *Sclerotinia bifrons* Whet. (*C. whetzelii*) and *Sclerotinia confundens* Whet. (*C. seaveri*), CUP 15624 and 17867 respectively, were examined.

No. 15624 contained abundant material of the same fungus we find around Ottawa.

No. 17867 consisted of two packets, one containing two dried cultures and the other containing a further two packets from the Herbarium of the New York Botanical Garden, labelled Fungi of Colorado No. 113 and No. 119. The latter consisted of leaves of the Rocky Mountain form of *Populus tremuloides* bearing sclerotia, some of which were detached or partially so. No. 113 consisted mainly of sclerotia similar to those in No. 119 bearing abundant apothecia, and mixed with leaf fragments and other debris. The apothecia agreed morphologically with the description by Whetzel (1950 (9)). The asci measured 46–65 × 6–7.5 μ and the ascospores 7–10 × 2.5–3.5 μ (Fig. 8*b*).

The packet containing dried cultures appears to have been included with the specimen in error. The cultures are dried on a piece of stiff paper on which is written in Whetzel's handwriting, "17867 = 5771 Phot'd. 12 day old cult. on Potato agar from sclerotial planting. Pl. 1-16-30. Dried. 1-28-30. Phot'd. Neg. c. conidia of Botrytis".

Examination of the cultures revealed that they are not a *Ciborinia*, but *Botryotinia fuckeliana* (De Bary) Whet. Whetzel (1940 (9)) made no mention of cultures in his description or discussion and had evidently recognized these as *Botrytis*. The most probable explanation seems to be that the western species, like the eastern, will not grow in culture and that Whetzel's plantings of the sclerotia became contaminated by air-borne spores of *Botrytis*, although it is remotely possible that sclerotia of *Botryotinia fuckeliana* were mixed in the collection and Whetzel used these by chance to make isolations. However, no evidence of any other *Botryotinia* sclerotia was found in the collection. Certainly the cultures in this packet are not the *Ciborinia*.

Seaver (1945 (7)) referred to a suggestion by Whetzel, apparently delivered verbally, that this fungus was not really a *Sclerotinia* but a *Helotium* parasitic on the sclerotia. Seaver rejected this suggestion and Whetzel apparently did not pursue it any further, but it must be admitted that the asci and ascospores (Fig. 8*b*) are much more suggestive of *Helotium* than of *Sclerotinia*. Seaver made a point of the fact that the apothecia were collected under trees in which sclerotia were evident in the living leaves, but to anyone familiar with the biology of the eastern species this is a highly suspicious circumstance. The question at once arises as to the source of the infection responsible for the sclerotia on the living leaves. In the east, *C. whetzelii* produces apothecia at the time the leaves are emerging and the period of fructification is relatively short so that by the time sclerotia are evident in the young leaves no apothecia can be found. There is no conidial state in *Ciborinia*; hence the sclerotia observed by Seaver and Shope in the living leaves must have come from ascospores, presumably discharged at the time the leaves were emerging. One would, therefore, expect that the period of fructification of the *Ciborinia* would be over by the time the sclerotia appeared on the leaves.

These biological considerations plus the fact that the asci and ascospores of Seaver's fungus are much more suggestive of *Helotium* than of *Ciborinia* make

it seem highly probable that Whetzel's surmise is true and that Seaver and Shope probably collected a *Helotium* growing on the old exhausted sclerotia of the *Ciborinia*.

Although Seaver and Shope did not have enough evidence to substantiate their claim that their fungus was the perfect state of the sclerotium on poplar, it is realized that we, also, do not have enough evidence to disprove it conclusively. The question can only be settled by intensive field work in Colorado. However, it is our opinion that when this work is done, it will be found that the ink spot disease of poplar in both east and west will be found to be caused by the same fungus, *C. whetzelii* (Seav.) Seav., and that the fungus here called *C. seaveri* will prove to be a *Helotium*. If it is transferred to *Helotium* the specific epithet "*bifrons*" should then be taken up for this fungus.

Ciborinia pseudobifrons Whetzel sp. nov. (Figs. 3, 4, 5, 6, 7, 8*c*).

Spermatia hyalina ovoidea, nata ex phialidibus fasciculatis.

Sclerotia ovata vel elongata, plana, atra, in texto folii bene firmata.

Apothecia late infundibuliformia vel ± applanata in maturitate, singula vel plura in sclerotio, 1–2(3) mm. diam. colore "avellaneous" (sensu Ridgway), pallidiora in juventute, longe stipitata; stipes 12 mm. vel brevior, tenuis coloris disci, fibrillosus ex pilis numerosis brevibus erectisque; hyphae rhizoideae desunt; asci cylindrici, ad basas attenuati, octospori, (100)110–150(180) × (6)7–9(10.5) μ; ascospori ovati, hyalini unicellulares, uniseriati, (7.5)9–13(16.5) × 3.5–5.0 μ; paraphysae tenues, ca 2μ diam. ad apicem paullum incrassatae ad 3.0–3.5 μ.

Habitat in *Populi* spp. foliis ramisquoque jacentibus.

Ciborinia pseudobifrons Whetzel sp. nov.

Spermadochia known only in culture, formed of minute tufts of fasciculate phialides. Phialides obclavate with the tip flaring and funnel-form. Spermatia hyaline, oval, with intercalary collar 2.0–3.0 × 1.5–2.5 μ.

Sclerotia firmly embedded in the partially decayed leaf tissue, usually numerous, oval or elongate, black, flat, up to 10–15 × 3–5 mm., usually smaller, in cross section showing a white medulla of very densely interwoven, relatively small hyphae, and containing embedded remnants of leaf-tissue, lacking palisade layer, outer layer of rectangular, dark-walled, rind cells, and covered by the firmly cemented, outer wall of the epidermal cells of the leaf.

Apothecia long-stipitate, one to several arising from any point on the face of the sclerotium, cups broadly funnel-form to flat-expanded when mature, fragile, 1–2(3) mm. in diameter, avellaneous (Ridgway), when young often very pale; stipe up to 12 mm. long, slender, concolorous with the cup, no rhizoidal hyphae at base, fibrillose with many erect, short hairs; asci 8-spored, long, slender, attenuated toward the base, (100)110–150(180) × (6)7–9(10.5)μ; ascospores uniseriate in the upper half of the ascus, ovate, hyaline, one-celled, non-guttulate, (7.5)9–13(16.5) × 3.5–5.0 μ; paraphyses slender, about 2 μ in diameter, the tips slightly enlarged to 3.0–3.5 μ.

On fallen leaves and twigs of *Populus* spp., leaves of *Ulmus* spp.

Type: CUP 16701 on leaves of *Populus tremuloides* Michx., McLean, N.Y. Collected by H. H. Whetzel and Cynthia Westcott.

Specimens examined: Kingsmere, Que., DAOM 5485, 26226. Tenaga, Que., DAOM 4593, 5492, 26264. Merivale, near Ottawa, Ont., DAOM 23251, 23252, 23253, 37559, 37572, 37575, 37576. South March, Ont., DAOM 37554, 37561, 37563, 37570, 37571.

Apothecia in culture, DAOM 26223, 26248, 26253.

Specimens cited by Whetzel in manuscript.

McLean, N.Y., CUP 16701, 16707, 15156, 15525, 15630, 15625, 26470, 26472, 25935, 14918. Labrador Lake, N.Y. CUP 15631, on unknown leaves. Tenaga, Que., DAOM 4593, CUP 26542.

The earliest and latest dates of collection among the Whetzel specimens are April 26 and May 27. The first collection was made on April 26, 1921, at McLean, N.Y. The collections in the Ottawa District have been made between April 24 and May 30.

The following is taken *verbatim* from manuscript notes of Prof. H. H. Whetzel.

"*Ciborinia pseudobifrons* appears to be an undescribed species. The only recorded species with which this might be confused is one described by Schroeter (1893 [(4)]) under the name of *Sclerotinia nervisequa* n. sp. The measurements given by Schroeter for the apothecia and ascospores of his species approximate those of *C. pseudobifrons*, but the dark colour of the apothecia, the smaller size of the asci and the nervisequent habitude of the sclerotia would seem to clearly distinguish it from our species. Although we have not been able to examine Schroeter's type material, a specimen on rotting leaves of *Alnus glutinosa*, distributed by Krieger (Fungi Saxonici 1486) under the name *S. nervisequa* Schroet. is certainly not identical with *C. pseudobifrons*. Schroeter records his species as occurring on the leaves of different broad-leaved trees, mentioning specifically collections on *Alnus glutinosa* and *Populus tremula*. *S. nervisequa* Schroet. is almost certainly to be referred to the genus *Ciboria* rather than to *Sclerotinia* or *Ciborinia*.

"It is interesting that *C. pseudobifrons* has almost always been collected from the foliage of *P. tremuloides* and under trees which are regularly infected by *C. bifrons*. [*C. whetzelii* (Seaver) Seaver][3]

"Apothecia are frequently to be collected at the same time under a given tree. The single exception known to the writer is his collection No. 15631 on unidentified leaves. However, *C. pseudobifrons* produces its apothecia over a much longer period. In fact, the apothecia of this species are usually more abundant toward the end or after the period of apothecial production by *C. bifrons* [*C. whetzelii*]. The constant association of these two species under the same tree, and the similarities of form and structure of their sclerotia and apothecia which are apparent, suggest that *C. pseudobifrons* may have been

[3] *In Whetzel's manuscript notes, C. bifrons refers to the species designated C. whetzelii in this paper.*

derived from *C. bifrons* [*C. whetzelii*]. Aside from its saprogenic habit it differs from *C. bifrons* [*C. whetzelii*] in its smaller apothecia arising usually several to many from the face as well as the margin of its embedded sclerotia, in the absence of rhizoids at the base of the apothecia, in the fibrillose or hairy character of the stipes of its apothecia, in its distinctly smaller asci and ascospores, and its much longer period of fruiting.

"There are also distinct differences in the sclerotia of the two species; those of *C. bifrons* [*C. whetzelii*] are concavo-convex, the medulla has a distinct and almost continuous palisade layer of large, rectangular, usually septate cells and the tissue remnants of the leaf embedded in the medulla are relatively sparse and scattered; those of *C. pseudobifrons* are flat or slightly bi-convex, a palisade layer in the medulla is wanting, the medullary hyphae are much smaller in diameter than those in the sclerotia of *C. bifrons* [*C. whetzelii*] and the leaf tissue remnants in the medulla abundant and striking."

Cultural Studies

An experimental series was begun on December 2, 1937 using, two mass and eight single ascospore cultures of *C. pseudobifrons*. The cultures were grown on wheat at 5° C. and 14° C. for about six weeks, then pieces of the wheat cultures about 2–3 cm. square were cut out and placed on moist, sterilized sand in dishes that were placed at 0° C. They were left at 0° C. for a month, then spermatized and placed at 5° C. Two spermatizations were carried out, one using spermatia from a single ascospore culture and the other using spermatia from a mass ascospore culture. A third set was unspermatized but was painted with sterile soil extract.

After spermatization all of the sand cultures were placed at 5° C. for two weeks, then transferred to 14° C. for 10 days and finally placed in the greenhouse shaded from direct sunlight on March 14, 1938.

In the dishes spermatized with spermatia from the mass culture, apothecia were produced in every isolate that was originally grown at 5° C. on wheat but only in seven of the isolates originally grown at 14° C. on wheat, and where they did develop, they were less abundant than in the isolates grown at 5° C.

In the dishes spermatized with spermatia from a single spore culture apothecia appeared abundantly in six of the cultures grown at 5° C., a few appeared in two others, and none appeared in the remaining two. In the cultures grown at 14° C. apothecia were abundant on one isolate, a few appeared on five others, and none appeared on four.

In the unspermatized dishes two of the cultures grown at 5° C. produced abundant apothecia, a few appeared on two others, and none appeared in six isolates. In the cultures grown at 14° C. a few developed in four isolates and none in the remaining six.

It was concluded that evidence of sexual strains existed. Since more than one isolate had been placed in each dish it was thought possible that spermatia could have been washed from one to the other while the dishes were being moistened. This would account for the appearance of apothecia in

unspermatized isolates. Apothecia were more abundant in the spermatized cultures and it was only where the mass culture was used for spermatia that apothecia appeared in all of them. It was obvious also from this series that better apothecium production could be obtained by growing the cultures at 5° C. rather than at 14° C.

A second experiment was set up using eight single ascospore cultures, three of which were obtained from apothecia in the previous series. These were carried through the same schedule as the cultures grown at 5° C. in the previous series. One set was left unspermatized and four spermatizations were carried out using single ascospore cultures as males. Each isolate was kept in a separate dish throughout the entire experiment.

The results are shown in Table I.

TABLE I

Ciborinia pseudobifrons, RESULTS OF CROSSES IN 1938 SERIES OF SINGLE ASCOSPORE CULTURES SHOWING CLEAR EVIDENCE OF SELF-STERILITY AND BIPOLAR REACTION

♂ \ ♀	54A SS3	54A SS4	54B SS2	54 SS3	54 SS4	54 SS7	54 SS10	54 SS11
54 SS3	+	−	+	−	−	−	+	+
54 SS4	+	−	+	−	−	−	+	+
54 SS7	+	−	+	−	−	−	+	+
54 SS10	−	+	−	+	+	+	−	−
Check unsperm.	−	−	−	−	−	−	−	−

After the apothecia in the compatible crosses had matured, half of the unspermatized dishes were spermatized with theoretically compatible isolates and in a little over a month apothecia appeared in all of these while the unspermatized ones remained sterile.

At this point we appeared to have clear evidence that the fungus was hermaphroditic, self-sterile, and bipolar, and no further work was done with it until 1952 when new series were undertaken with fresh isolates.

Single and mass ascospore cultures of two collections were used. No. 52–54 was from an apothecium on a single sclerotium with the host unknown, and No. 52–73 was from an apothecium on a catkin, thought to be either *Populus* or *Salix*. These apothecia were not identified when first collected, but from the appearance of the cultures and the morphology of the apothecia produced in culture it was concluded that they were *Ciborinia pseudobifrons*. The results of the crosses are shown in Table II.

TABLE II

Ciborinia pseudobifrons RESULTS OF CROSSES IN TWO 1952 SERIES OF MASS AND SINGLE ASCOSPORE CULTURES SHOWING EVIDENCE OF SELF-FERTILITY

♂ \ ♀	52-54					♂ \ ♀	52-73				
	Mass	SS1	SS2	SS3	SS4		SS1	SS2	SS3	SS4	SS5
52-54						52-73					
Mass	+	+	+	+	+	Mass	+	+	+	−	+
SS2	+	−	−	+	+	SS1	+	+	−	−	+
SS3	+	+	+	+	+	SS2	+	+	+	+	+
SS5	+	+	+	−	−	SS3	−	+	−	−	+
Check	+	+	−	−	+	Check	+	+	+	−	+

Although apothecia did not appear in every dish, they did appear in selfed and unspermatized dishes and as every culture was left in a separate dish throughout the experiment, clear evidence was provided that these two isolates are homothallic and self-fertile.

The following year more intensive collecting was done and a number of fresh isolates were obtained that were identified as *Ciborinia pseudobifrons*. Another series was carried out using seven single ascospores from fresh collections and three from apothecia produced in the previous series.

The fresh isolates were as follows:

481—on twig of *Populus grandidentata*
482—on a sclerotium, substrate unknown
483—on leaf of *Populus*
485—on fragment of unidentified leaf, probably not *Populus*
486—on leaf of *Ulmus*
487—on unidentified leaf
488—on a sclerotium, substrate unknown
489—on a leaf of *Acer* ?

These collections were also identified on the basis of the size of asci and ascospores and the appearance of the cultures.

The results of this series are shown in Table III.

In this series also, the results indicated that these isolates were homothallic and self-fertile. These findings are in marked contrast to the clear indication of self-sterility and bipolarity obtained in the earlier series.

In 1954 a further series was carried out using nine isolates from fresh collections of that spring. These included collections on twigs of *Populus*, leaf of *Ulmus*, and unidentified leaf fragments. In this series some difficulty was experienced with air contaminants but again the results indicated that these isolates were homothallic and self-fertile.

TABLE III

Ciborinia pseudobifrons RESULTS OF CROSSES IN 1953 SERIES OF SINGLE ASCOSPORE CULTURES SHOWING EVIDENCE OF SELF-FERTILITY

♂ \ ♀	481 SS2	483 SS1	485 SS1	486 SS1	487 SS2	488 SS1	489 SS1	F-2 SS2	R SS1	L-9 SS1
482 SS1	−	+	+	+	+	+	+	−	+	+
482 SS2	−	−	+	+	+	+	+	+	+	−
488 SS1	−	+	+	+	+	+	+	+	+	+
L-9 SS2	−	+	+	+	+	+	−	+	+	−
Check	−	−	+	+	+	+	−	−	+	+

Obviously further work needs to be done with this species to determine whether or not we may be dealing with two species, or self-fertile and self-sterile strains within the species, and whether or not these are related to host substrate. However, since the junior author is obliged to discontinue this work it was thought desirable to record the results obtained so far even though they are inconclusive.

Ciborinia davidsoniana Whetzel sp. nov. (Fig. 8*d*)

Sclerotia irregularia, orbicularia sine angularia, plana, atra, bene firmata in texto folii.

Apothecia leniter patellaria, singula vel geminata in sclerotio, 0.5–1.5 mm. diam., rubro-lutea in juventute, rubro-brunnea in aetate, extus obscuriora, luteo-brunnea vel rubro-brunnea, stipitata; stipes elongatus, tenuis, atro-brunneus vel ater ad basas, interdum paullum incrassatus sparse pruinosus ex hyphis albis; asci cylindrici, attenuati ad basas, octospori 135–153 × 6.75–8.25 μ; ascospori hyalini, elliptici aequilaterales vel ± inaequilaterales, uniloculares, uniseriati, (7)9–12.3 × (3)4.5–6 μ; paraphysae tenues 2.5–4.5μ diam.

Habitat in *Populi tremuloidis* foliis hiematis.

Ciborinia davidsoniana Whetzel sp. nov.

Sclerotia crustiform, definite in outline, irregular, circular or angular, large or small [about 2–4 mm.][4], black, flat, scarcely thicker than the leaf blade, involving large as well as small veins, firmly embedded in the leaf tissue.

Apothecia one or two from each sclerotium, shallow saucer-shaped, 0.5–1.5 mm. diam., disk reddish-yellow when young becoming reddish-brown with age,

[4] *Notes in brackets in this description have been added by the authors to Whetzel's manuscript notes.*

externally darker, yellowish-brown to reddish-brown, stipe long, slender, dark brown to black near the base, sometimes slightly swollen, sparsely pruinose with white hyphae; asci [cylindric, tapering to a slender stalk, eight-spored] usually collapsed when spores are mature leaving the spores as if in chains of eight, 135–153 × 6.75–8.25 μ; ascospores [hyaline, one-celled, elliptical or somewhat inequilateral, uniseriate], (7)9–12.3 × (3)4.5–6 μ; paraphyses [hyaline, filiform], branched only near the base, 2.5–4.5 μ diam.

Host: on overwintered leaves of *Populus tremuloides* (probably var. *aurea*).

Type: CUP 35200. Above range stations, Buzzard Creek, Grand Mesa National Forest, Colorado. June 24, 1930. Coll. R. W. Davidson No. 421.

The material in this collection is rather scanty. One mount was made from it which proved rather unsatisfactory, but confirmed the ascus and spore sizes in Whetzel's notes, except that the spores were slightly smaller and the asci slightly narrower than he indicated. However, it seemed desirable to publish this description and possibly stimulate collectors in that area to search for fresh material of the fungus.

The second unpublished species in Whetzel's notes was labelled *Ciborinia populicola*, CUP 25237. This specimen consisted of two packets, one of leaves of *Populus nigra* L. var. *italica* Du Roi bearing sclerotium-like structures, and the other containing apothecia produced in culture from isolates from the above sclerotia.

Microscopically these apothecia are indistinguishable from *Ciboria acerina* Whet. & Buchw. The asci are four-spored with quite a number of two- and three-spored ones. They agree with those of *C. acerina* in size as do also most of the spores. Some of the spores are larger but these seem to come from the two- and three-spored asci.

The apothecia have very short stipes and are broader and more robust than those of *C. acerina* as usually found in nature, but they match very well in stature apothecia of *C. acerina* developed in culture at Ottawa (DAOM 19518 and 28501). There is nothing in Whetzel's notes on the cultures to exclude *C. acerina*; in fact the notes rather suggest this species, and the stromata are not more sclerotium-like than is sometimes found in *C. acerina*. It is known that this species occurs on a number of plants other than *Acer* and we are of the opinion that this specimen is simply *C. acerina* on *Populus*.

Acknowledgments

The authors are especially indebted to Dr. R. P. Korf for the loan of manuscript notes and specimens of the late Professor H. H. Whetzel. Dr. F. L. Drayton carried out some of the early work on *Ciborinia pseudobifrons* and has maintained continued interest in the problem and critically read the manuscript. Dr. B. Boivin provided the Latin diagnoses. Helpful suggestions concerning the nomenclatural problem have been received from discussion with Dr. Boivin and Dr. R. A. Shoemaker and in correspondence with Dr. Korf, Dr. D. P. Rogers, and Dr. R. W. G. Dennis.

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SCLEROTINIA BOREALIS IN CANADA[1]

By J. Walton Groves and Constance A. Bowerman[2]

Abstract

Sclerotinia borealis Bubak & Vleugel, previously known only from Sweden, Norway, Finland, and the U.S.S.R., has been found causing injury to cultivated grasses at Prince George, B.C. Apothecia have been developed in culture and compared with authentic material from Sweden. The fungus is homothallic and self-fertile.

Sclerotinia borealis Bubak & Vleugel is a fungus known to attack various species of forage grasses and cereals, sometimes causing serious injury. Jamalainen (1949 (2)) gave an account of the fungus including its host range. Apparently it has been recorded so far only from Sweden, Norway, Finland, and the U.S.S.R.

In 1951 Dr. M. W. Cormack, Science Service Laboratory, Lethbridge, Alta., sent specimens of sclerotia to Dr. F. L. Drayton suggesting that they might be of this fungus. They had been collected in the experimental plots of grasses at Prince George, B.C., and were observed to be associated with severe injury of a snow mold type.

Specimens were received on the following grasses: *Agrostis canina* L., *Agropyron dasystachum* (Hook.) Scrib., *A. desertorum* (Fisch.) Schult., *A. intermedium* (Host) Beauv., *A. semicostatum* (Steud.) Nees ex Boiss., *A. sibiricum* (Willd.) Beauv., *Alopecurus pratensis* L., *Arrhenatherum elatius* (L.) J. & C. Presl, *Bromus erectus* Huds., *B. inermis* Leyss, *Dactylis glomerata* L., *Elymus canadensis* L., *E. sibiricus* L., *Festuca elatior* L., *F. gigantea* (L.) Vill., *F. rubra* L., *Lolium perenne* L., *Phleum pratense* L., *Poa ampla* Merr., and *P. compressa* L.

An investigation of the fungus was thereupon undertaken, based on this material, with the object of determining whether or not the same fungus was attacking the various grasses and, if possible, to develop apothecia in culture and make certain of the identity of the species.

The fungus was isolated by surface sterilizing the sclerotia in a solution of commercial Javex adjusted to 2% available chlorine and plating them on potato dextrose agar. It is noteworthy that when isolations were attempted at room temperature no growth occurred. Development of mycelium took place only when the plates with surface-sterilized sclerotia were incubated at 10° C. or lower. The isolates from different grasses appeared to be identical in every respect.

The procedure followed in attempting to develop apothecia in culture was essentially the same as that described by Drayton (1937 (1)). The cultures were grown on sterilized wheat grains in the dark at 5° C. for three months.

[1] *Manuscript received June 27, 1955.*
Contribution No. 1470 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] *Principal Mycologist and Assistant Mycologist respectively.*

The sclerotia were then removed and placed on moist, sterilized, quartz sand in the dark at 0° C. for two months. They were then spermatized and placed in the dark at 5° C. for one month and transferred to the greenhouse, shaded from direct sunlight, and maintained at a temperature near 10° C. but with possible fluctuations of about 5° C.

Mature apothecia were developed in about two months. They appeared in crossed, selfed, and unspermatized cultures and, since each isolate was kept in a separate dish throughout the entire experiment, it can be confidently stated that the fungus is homothallic and self-fertile. It is a *Sclerotinia sensu stricto* because it lacks a conidial state and the apothecia arise from a tuberoid sclerotium.

The asci and spores agreed well with the description of *S. borealis* but it was felt that it was desirable to compare our material with authentic specimens from Sweden. Through the kindness of Professor J. A. Nannfeldt and Dr. H. Ekstrand we received specimens of dried apothecia and a culture.

According to Professor Nannfeldt, the dried specimens were collected by the Danish mycologist Lind during a visit to his friend Vleugel in Luleå, North Sweden. This is the type locality and the material was probably collected in Vleugel's company. Apparently only the sclerotia were collected and the apothecia were developed from them after Lind's return to Lyngby.

The asci and spores in these specimens agreed very well with those in our material although the spores in both the Swedish and Canadian specimens were slightly smaller than the size stated in the original description. The apothecia in the Swedish specimen were smaller and more delicate than those developed in our cultures, but we have observed in other species of Sclerotiniaceae that apothecia produced in culture are usually more robust than those formed in nature.

The culture received from Dr. Ekstrand appeared to be the same as the Canadian isolates. Another culture series was set up using both the Swedish and Canadian isolates. Apothecia were produced, but in this series they failed to produce mature spores in any of the isolates. As this is a low temperature fungus it is possible that the greenhouse temperature may have become too high at some stage, but the actual reason for the failure is not known. However, the apothecia in the Swedish isolate were as robust as those in the Canadian isolates and agreed with them in appearance and stature.

The Canadian fungus is, therefore, considered to be *Sclerotinia borealis* Bub. & Vleug. and the following description is based on the Canadian material.

Sclerotinia borealis Bub. & Vleug. *In* Vleugel, Svensk Bot. Tidskr. 11 : 308. 1917. Figs. 1–4.

Sclerotia tuberoid, variously shaped, usually more or less elongated, oblong to nearly circular, sometimes curved or lobed, mostly 3–8 mm. long by 2–4 mm. broad, black, easily detached from the substrate.

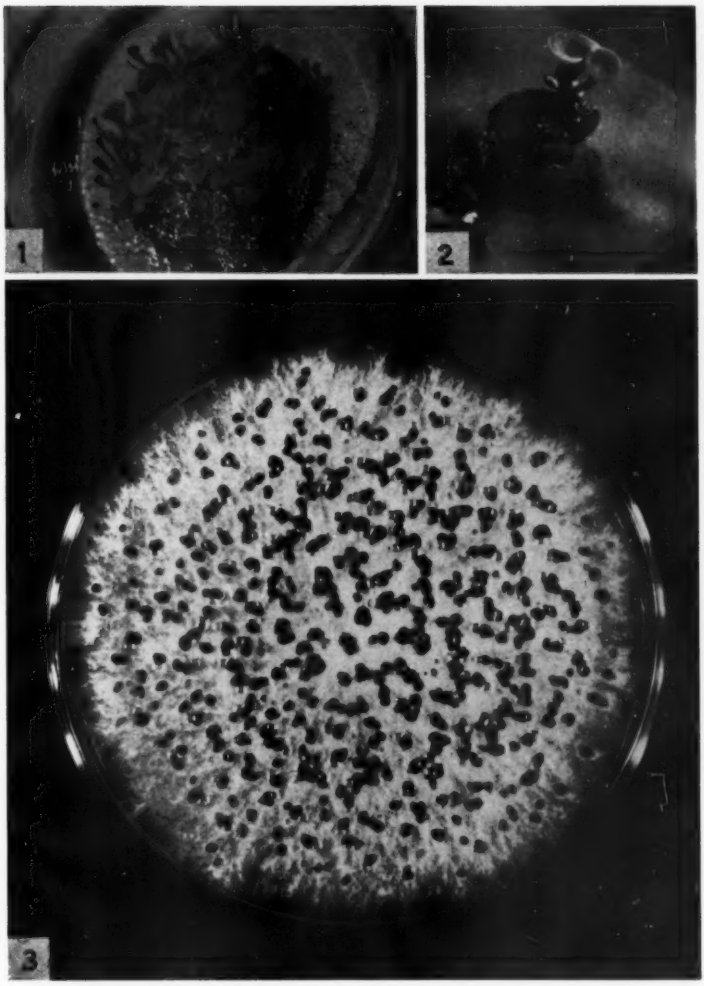

FIG. 1. Apothecia of *S. boralis* developed in culture on sand in a culture dish.
FIG. 2. Cluster of apothecia removed from the dish.
FIG. 3. Culture of *S. borealis* on potato dextrose agar grown at 10° C., five weeks old.

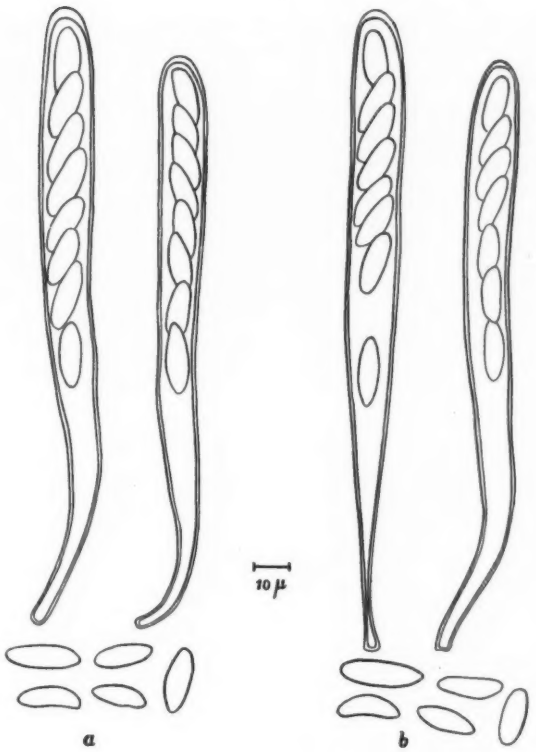

FIG. 4. *a*. Asci and ascospores of *S. borealis* from Swedish specimen. *b*. Asci and ascospores from apothecia produced in culture.

Spermatia known only in culture, hyaline, globose, 2.5–3.5 μ diam., produced endogenously in flask-shaped phialides borne in clusters on the mycelium.

Conidia lacking.

Apothecia arising from the sclerotia singly or in groups, stipitate, cyathiform, expanding to shallow cup-shaped, 2.5–5.5 mm. diam., "bister" to "Natal brown" (Ridgway), hymenium even or umbilicate-depressed, fleshy, margin even or sometimes irregular; stipe 2–6 mm. long, about 1 mm. diam. at the apex, tapering slightly toward the base, paler than the hymenium, finely pubescent above to subtomentose at the base; tissue of the hypothecium and stipe prosenchymatous, composed of ascending, more or less parallel to interwoven, hyaline to faintly yellowish hyphae, (4) 6–8 (13) μ diam., curving toward the outside and forming a more compact excipular zone, appearing pseudoparenchymatous, with short, hyaline hairs arising from the outer cells; subhymenium a narrow, compact zone of closely interwoven,

slender, yellowish hyphae about 3 μ in diameter; asci cylindric, tapering below to a long, slender stalk, eight-spored, 175–200 (238) $\times$ 11–14 μ; ascospores hyaline, one-celled, ellipsoid to ellipsoid–fusiform, sometimes slightly curved or unequal sided, uniseriate, (15) 17–21 (25) $\times$ 6–8 μ; paraphyses hyaline, filiform, septate, simple, 2–3 μ in diameter, the tips sometimes slightly enlarged.

Host: species of Gramineae.

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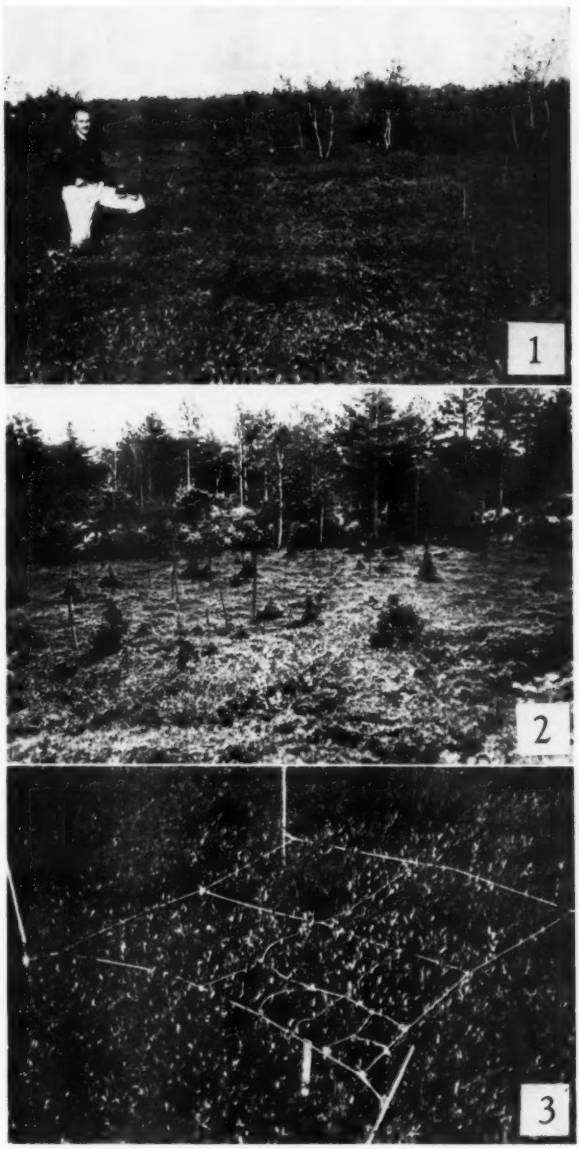

FIG. 1. Large stand of *Chamaedaphne calyculata* at Farnham, Que.
FIG. 2. First step of the invasion of a *Chamaedaphne* stand by *Picea mariana*.
FIG. 3. Frame used for field work.

STUDIES IN FOREST PATHOLOGY

XV. ROOTLETS, MYCORRHIZA, AND SOIL TEMPERATURES IN RELATION TO BIRCH DIEBACK[1]

By D. R. Redmond[2]

Abstract

Cylindrosporium sp., isolated from dead yellow birch roots, inhibited the growth of another fungus isolated from mycorrhiza and dead roots. Growth rate of the former increased while that of the latter decreased with increased temperature above the optimum for rootlet development. Twenty-four species of fungi were isolated from mycorrhiza. Seedlings grown aseptically in soil and in agar were inoculated with fungi isolated from dead and dying rootlets and from mycorrhiza, but none of the organisms displayed sufficient virulence to cause birch dieback. The optimum temperature for development of potted seedlings was about 18.5° C. Increases or decreases of 2° C. or more from the optimum for yellow birch development resulted in increased rootlet mortality, decreased frequency of mycorrhiza, and a thinner mantle of hyphae in mycorrhiza, followed by reduction in foliage vigor. Normal rootlet mortality of 6% in a 55-year-old yellow birch stand was increased to 19 and 60% by raising soil temperature 1° and 2° C., respectively, above the normal during one summer. Soil moisture was not lowered a statistically significant amount by a 5° C. increase until the sixth day following rain. Mycorrhiza existed on residual living roots in about the same proportion as on undisturbed root systems. The possibility that birch dieback may be the result of soil temperature increases is discussed. It is suggested that cutting practices in hardwood stands should remove all yellow birch trees or prevent increased temperatures in soil occupied by residual birch.

Introduction

Yellow birch (*Betula lutea* Michx. f.) and white birch (*Betula papyrifera* Marsh.) until recently comprised a large proportion of the hardwood forests in Canada, east of Ontario, yellow birch being the most valuable species. Statistics to show the volume of these species cut annually are not available for this region, but those for Nova Scotia are quite indicative of the high economic value of birch to the hardwood lumber industry. During the 30-year period 1921 to 1950 inclusive, the total volume of hardwood cut for sawlogs and veneer in that Province was 518 million bd. ft., 68% of it being yellow birch and 11% white birch.* The recognition of low vigor in undisturbed birch stands about two decades ago therefore caused considerable concern. Since that time most of the merchantable birch has disappeared from the forests of New Brunswick and Nova Scotia and canopies of many stands formerly composed mainly of yellow birch appear as that shown in Fig. 19.

The first symptom of decline is a thinning of the foliage accompanied by small, sometimes chlorotic or curled, leaves at the tips of shoots in the

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Contribution No. 177 from the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada. Based on a thesis presented in June, 1954, to the Graduate School, Yale University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
[2] *Officer-in-Charge, Forest Pathology Investigations, Forest Biology Laboratory, Fredericton, New Brunswick.*

* *Information supplied in correspondence by L. J. Pouliot, Forestry Section, Bureau of Statistics, Ottawa, Canada.*

periphery of the crown. Later, twigs become bare because buds die or fail to open and the foliage grows thinner in the interior of the crown. Still later, branches and whole portions of the crown die, the foliage remaining is bunched because of shortening of internodes, and it is usually confined to lower branches and adventitious shoots (Figs. 20, 21, and 22). Death of trees, when it occurs, takes place within three to five years after the initial appearance of symptoms.

Dying of birch had been reported in cutover lands (11, 23) but it was not until about 1932 that widespread decline in vigor was reported in undisturbed stands (2). This was first observed in New Brunswick and each year the condition became worse until mortality rates of yellow birch as high as 90% had occurred in some areas and dying of trees was reported from Nova Scotia, Quebec, and Maine (1, 16, 18, 19).

Primary studies of the cause of dieback showed that the bronze birch borer (*Agrilus anxius* Gory), the only insect associated in sufficient numbers with dying trees to be capable of initiating damage, was not responsible (2, 13, 16). Attempts to isolate a fungus that might have initiated dieback in crowns of trees have been unsuccessful (14) and at the present time there are no reports of presence of a virus that can be considered the basic cause (12). It has been stated that the cause may be a change in climatic conditions (2, 13, 14), and a reduction in growth rate prior to the occurrence of advanced deterioration was reported by several workers (2, 16, 18). Studies of ecological and physiological conditions in birch showed that no relation existed between the occurrence of dieback and site condition, but suppressed trees were less affected (7) and it was reported that the first trees to die were those on dry, shallow-soiled areas (18). The water content of aerial portions of individual trees decreased as deterioration advanced, but no temporary or permanent mechanical stoppages in the water-conducting tissues could be located in trees in any stage of dieback (9). It was shown that moisture content of aerial portions of yellow birch was low during one drought year (1949) when soil was deficient in moisture (5).

Excavation of root systems of trees conducted over a period of years showed that the number of dead rootlets increased as deterioration in the crown progressed, and it was stated (15) that " . . . the first symptoms of dieback were in the form of dead rootlets." It was suggested (8) that the moribund condition of root systems of trees showing crown deterioration may constitute a factor of great importance in normal restocking of the water supply in trees after it had been depleted by normal transpiration.

There are no reports of studies being conducted on the fungi associated with rootlets of yellow birch trees and it was to discover the possible presence of any root parasite that the following investigation was made.

Fungi Isolated from Dead and Dying Rootlets of Yellow Birch

During the summer of 1950, 3000 isolations were made from dead and dying rootlets of 176 yellow birch trees in Nova Scotia. The number of trees

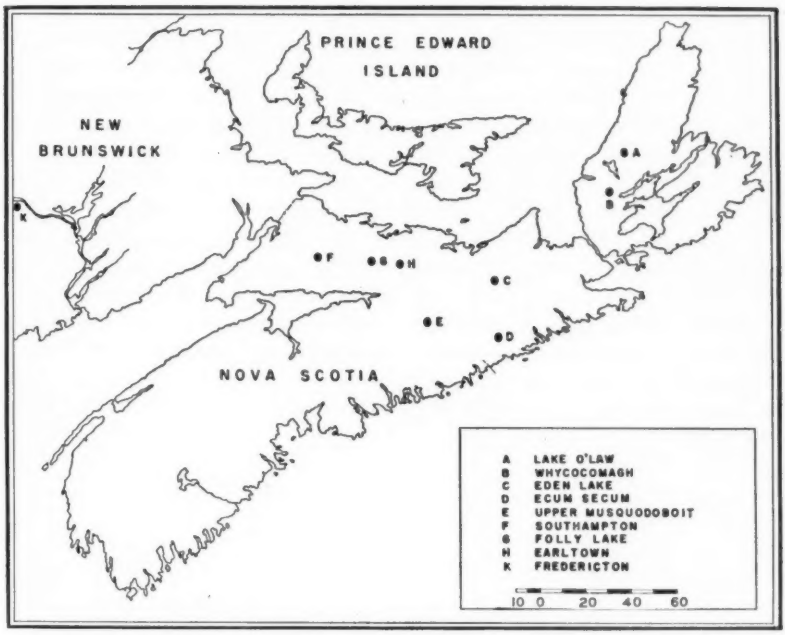


FIG. 1. Map of Nova Scotia and southeastern New Brunswick showing locations where samples of living and dead rootlets of yellow birch were collected for culturing associated fungi.

and the locality of sampling was as follows: 107 at Lake O'Law, 11 at Whycocomagh, 10 at Eden Lake, 10 near Ecum Secum, 10 at Upper Musquodoboit, 10 at Southampton, 10 at Folly Lake, and 8 at Earltown (Fig. 1). From 143 of the trees 222 isolations were made from the advancing margins of lesions in roots. Samples of rootlets from which isolations were made were submerged and washed in 0.01% solution of mercuric chloride for one of the following periods of time: 15 sec., 30 sec., 1 min., 2 min., or 3 min., rinsed in three changes of sterile distilled water, and placed aseptically on 4.5% Difco malt-agar in Petri plates.

Twenty-four species of fungi were isolated from dead and dying rootlets. Five species of Phycomycetes, five *Penicillium* spp., two *Aspergillus* spp., and *Trichoderma viride* Pers. were commonly isolated when the rootlets were not sterilized, when sterilization was of short duration, or when rootlet samples were badly decomposed. Two minutes of sterilization in 0.01% mercuric chloride solution was usually sufficient to kill all organisms on the surface of rootlets. Four fungi commonly isolated from the xylem of recently dead (sometimes dying) rootlets were: *Mortierella alpina* Peyronel, *Cephalosporium* sp., *Cylindrosporium* sp., and an as yet undetermined species designated as

"Unknown B."* *Epicoccum purpurascens* Ehrenb., *Phomopsis* sp., and six other as yet undetermined species were found occasionally. Although no fungus was universally present in the advancing margins of lesions in dying roots, the incidence of *Cylindrosporium* sp. and "Unknown B" was relatively high.

The only fungus found with any consistency in association with dead and dying rootlets was *Mucor varians* Povah, and it was isolated only when surface sterilization was lacking or of short duration. Thus there was no evidence to indicate that any specific fungus was responsible for birch dieback (Table I).

Examination of Table I shows that no consistent relationship existed between the occurrence of any of the species of fungi isolated from the rootlets and the health of foliage in the crown. Differences in their occurrences seemed to depend, in some cases, upon the geographical region from which the sample was taken (Table II). For example, *Absidia glauca* Hagem was not isolated on Cape Breton Island but was found rather commonly on the Nova Scotia mainland, whereas "Unknowns M, U, and Z" and one species of *Penicillium* were found on Cape Breton Island only.

TABLE I

PERCENTAGE OF TREES IN EACH CROWN INJURY CLASS FROM WHICH EACH FUNGUS WAS ISOLATED

Fungus	Description of crown injury: Normal	Dead twigs due to mechanical injury	Foliage weak but inconspicuously so	As 2A but decidedly so	Bare twigs, weak foliage, but no dead branches	Few bare twigs, foliage normal	Dead branches but less than half the crown	Over half of crown dead	No living branches except adventitious	Tree recently dead, cambium dry at 4 ft.	All crown injury classes
No. of trees	26	41	10	21	12	1	28	24	12	1	176
Phycomycetes											
Absidia glauca Hagem	62	27	—	—	8	—	4	4	25	100	19.3
Endogone sphagnophila Atk.	15	17	—	10	8	—	14	13	17	—	13.1
Mortierella alpina Peyronel	35	44	50	57	33	—	39	50	25	—	39.2
Mucor ramannus A. Moeller	12	7	—	10	17	—	11	13	—	—	9.1
Mucor varians Povah	96	100	90	100	100	100	100	96	92	100	97.7
Other fungi											
Aspergillus spp.	—	7	—	10	17	—	—	—	8	—	4.5
Cephalosporium sp.	8	5	30	—	8	—	11	4	—	—	6.8
Cylindrosporium sp.	58	44	20	—	42	100	54	63	67	100	48.9
Epicoccum purpurascens Ehrenb.	—	2	—	5	—	—	7	—	—	—	2.3
Penicillium spp.	4	22	30	5	25	100	18	13	33	—	17.0
Phomopsis sp.	—	5	—	5	—	—	18	4	17	100	6.8
Trichoderma viride Pers.	15	12	—	10	25	—	14	—	17	—	11.4
"Unknown B"	50	63	90	95	75	100	79	58	67	—	69.3
"Unknown M"	4	2	30	14	8	—	4	—	17	—	6.8
"Unknown N"	—	2	—	19	8	100	7	4	8	—	5.7
"Unknown P"	8	10	20	5	33	—	7	4	17	—	9.7
"Unknown Q"	4	7	—	5	—	—	14	4	—	—	5.7
"Unknown U"	—	—	—	5	8	—	—	—	—	—	1.1
"Unknown Z"	4	5	10	—	17	100	7	—	—	—	5.1

** Several of the fungi isolated have not been identified, although cultures have been studied by mycologists who specialize in the Fungi Imperfecti. Many of these forms do not sporulate in culture. Different species are referred to here as unknowns and letters are used to differentiate them.*

TABLE II

PERCENTAGE OF TREES FROM WHICH FUNGI WERE ISOLATED, BY GEOGRAPHICAL LOCATION

	Geographical location							
	Lake O'Law	Whycocomagh	Eden Lake	Upper Musquodoboit	Ecum Secum	Southampton	Folly Lake	Earltown
No. of trees	107	11	10	10	10	10	10	8
Phycomycetes								
Absidia glauca Hagem	—	—	—	40	—	90	100	100
Endogone sphagnophila Atk.	2	—	—	40	80	20	50	25
Mortierella alpina Peyronel	61	20	—	10	10	—	30	20
Mucor ramannianus A. Moeller	11	30	30	—	—	—	—	—
Mucor varians Povah	97	90	100	90	90	100	100	60
Other fungi								
Aspergillus sp.	8	10	—	—	—	10	—	—
Cephalosporium sp.	10	10	—	—	—	—	—	—
Epicoccum purpurascens Ehrenb.	3	—	10	—	—	—	—	—
Penicillium spp.	18	20	50	10	30	—	10	—
Phomopsis sp.	1	—	—	30	60	20	—	—
Trichoderma viride Pers.	8	20	50	10	—	10	20	—
"Unknown B"	81	80	90	70	60	50	30	25
"Unknown M"	11	—	—	—	—	—	—	—
"Unknown N"	9	—	10	—	—	—	—	—
"Unknown P"	5	70	20	—	—	10	—	25
"Unknown Q"	6	—	—	—	20	—	10	10
"Unknown U"	2	—	—	—	—	—	—	—
"Unknown Z"	7	10	—	—	—	—	—	—

Fungi Associated with Living Rootlets of Yellow Birch

As early as 1947 improvement in foliage conditions of yellow birch was noticeable in some areas, by 1950 it was common, and by 1951 many trees that had lost even more than half their crowns were producing very healthy and normal looking foliage as shown in Fig. 23. Improvement by 1952 was widespread throughout the Maritime Provinces. In 1951 examination of root systems of yellow birch in Nova Scotia and New Brunswick revealed mycorrhiza on almost all living rootlets, a sharp contrast with conditions existing in trees that were suffering dieback during 1947 and 1948. A survey was undertaken to determine the fungi associated with mycorrhiza, and also whether these were the same species that had been isolated previously from dead and dying rootlets.

Fungi from mycorrhiza were isolated on yeast extract agar in slant tubes and, as soon as growth of a fungus was well established on the medium, hyphal

tips were transferred aseptically to a 125-ml. Erlenmeyer flask containing sections of small yellow birch roots (Fig. 25) that had been steam-sterilized at 100° C. for 90 min. A total of 1683 isolations was made from all the areas where sampling had been conducted during 1950 in Nova Scotia (Fig. 1) and 1329 from the vicinity of Fredericton, New Brunswick. It was found that each tree sampled had associated with it several species of fungi and in many cases an individual mycorrhiza contained more than one fungus. During the survey period, beginning in June, 1951, it was noted, as the summer progressed and the soil grew warmer, that fewer fungi could be isolated from mycorrhiza. By August very few attempts were successful. Sampling was extended throughout the winter and attempted isolations were much more successful after soil temperatures had decreased and autumn rains had begun in October. When the soil became colder, different fungi were found and mycorrhizal structures that were absent or rare in summer became frequent. Of 26 species of fungi isolated only one, "Unknown B", was recovered throughout the year, 8 species were found only during the winter months, only 4 were isolated during August, and the remaining 13 were isolated during May, June, and July. It was apparent also that root growth occurred throughout the winter, but to a limited extent only. This was indicated by the common occurrence of mycorrhiza of the type shown in Fig. 26, which is believed to be formed when periods favorable for root growth are intermittent (17). This structure was not found during summer, except during an experiment to be described later, when it occurred in areas where the soil temperature was limiting and mortality of rootlets caused by increased soil temperature was only slightly above normal.

Antagonism Between Fungi Isolated from Yellow Birch Rootlets

Although *Mucor varians* was the only fungus isolated from dead and dying rootlets of nearly all trees, *Cylindrosporium* sp. and "Unknown B" occurred quite commonly, as did also *Mortierella alpina*, which has been shown to form endotrophic mycorrhiza with higher plants. Also, the presence of *Cephalosporium* sp. was potentially significant since several species of *Cephalosporium* are known to be virulent parasites on forest trees. For these reasons it seemed advisable to determine the effect of these organisms on one another and on rootlets of healthy seedlings.

Since it has been suggested that changes in climate (2), especially increased temperature during the growing season (14), might be the primary cause of dieback, a series of laboratory experiments was set up to determine the optimum temperature and pH requirements of *Cephalosporium* sp., *Cylindrosporium* sp., *Mortierella alpina*, and "Unknown B". Liquid medium consisting of 20 gm. of glucose and 5 gm. of Difco dehydrated yeast extract per 1000 ml. of water was used in all these tests. Cultures were incubated in 10 ml. of medium in 125-ml. Erlenmeyer flasks for 14 days on a reciprocating shaker at 104 complete oscillations per minute. The amount of fungus material in each aliquot of inoculum was calculated to be between 0.03 and 0.05 mgm.

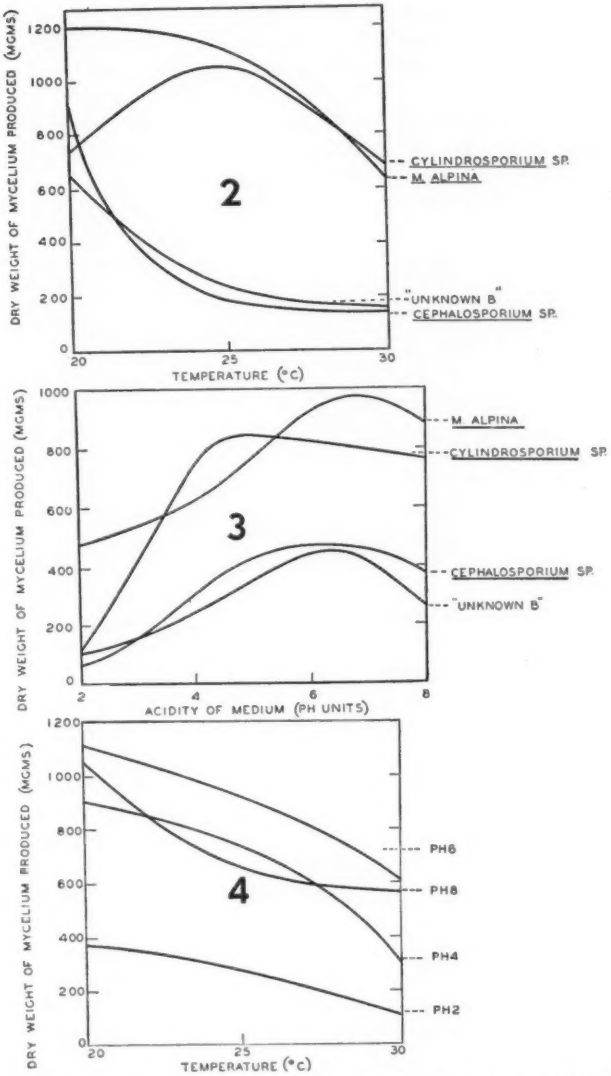


FIG. 2. Mycelium production by "Unknown B", *Cephalosporium* sp., *Cylindrosporium* sp., and *M. alpina* after the effect of pH had been removed, as affected by temperature changes.

FIG. 3. Mycelium production by "Unknown B", *Cephalosporium* sp., *Cylindrosporium* sp., and *M. alpina* after the effect of temperature had been removed, as affected by hydrogen ion concentration.

FIG. 4. Combined production of mycelium by "Unknown B", *Cephalosporium* sp., *Cylindrosporium* sp., and *M. alpina* at different temperatures as related to hydrogen ion concentration of the media.

At the end of the experiment two types of measurements were made from each culture: the oven-dry weight to the nearest 0.1 mgm. of mycelium produced, and the final pH of the medium from the filtrate, after the fungus had been removed.

Figs. 2, 3, and 4 show that there were striking differences in the growth rates of the fungi at different temperatures and with variations in pH values. When the values for growth rates of each fungus at pH 2.0, 4.0, 6.0, and 8.0 were combined it was shown (Fig. 2) that "Unknown B", *Cephalosporium* sp., and *M. alpina* grew more slowly at temperatures above 20° C., but the growth rate of *Cylindrosporium* sp. increased as temperature was increased to 25° C. When the differences between the effect of temperature at 20° C., 25° C., and 30° C. were eliminated, the optimum pH value appeared to be about 6.0 (Fig. 3) for all fungi except *Cylindrosporium* sp., which seemed more favored by slightly lower values, about 4.0 to 5.0. When the growth of all fungi was combined (Fig. 4), it was shown that pH 6.0 provided the most favorable and pH 2.0 the least favorable conditions at all temperatures, and pH 8.0 was more favorable at 20° and 30° C. than pH 4.0, but the reverse was true at 25° C. Statistical analyses of the growth rates of these fungi showed that *M. alpina* and *Cephalosporium* sp. each grew significantly faster at 20° C. than at higher temperatures, that within the range studied temperature produced no significant change in the growth rate of *Cylindrosporium* sp., and that *Cylindrosporium* grew significantly faster than "Unknown B" at 25° C. A statistical analysis of the growth rates as affected by variations in pH values between 4.0 and 8.0 showed that alterations of the acidity of the medium between these extremes did not have nearly as much effect on growth rate as was induced by an increase of 5° C. above the soil temperature occurring normally under a closed yellow birch stand, where pH values between 4.5 and 5.5 are most common. Fig. 5 shows that temperatures at rooting depth under yellow birch range from 5° C. to 22° C. during the growing season, but temperatures above 20° C. may be considered as occurring only infrequently and briefly. Therefore, it is concluded that variations in temperature that could occur in nature might exert far greater alterations in the physiological behavior of these fungi than any changes in acidity that would be likely to occur.

The fungi were also grown individually and in all combinations in liquid medium in such a way that there would be free interchange of all ingredients in the medium and all products of fungus metabolism between individual colonies in a flask, but no mixing of the mycelium of one colony with that of another. Thimbles of finely porous alundum were used to separate the organisms and the experiment was run at 25° C. for 10 days. From the data presented in Table III it is readily apparent that *Cylindrosporium* sp. completely inhibited growth of *M. alpina* and *Cephalosporium* sp., and partially inhibited "Unknown B". Also, "Unknown B" completely inhibited all but *Cylindrosporium* sp. *Cephalosporium* sp. reduced growth of *M. alpina* by about 25%, whereas *M. alpina* reduced growth of *Cephalosporium* sp. by more than 50%.

It was thought that the inhibiting substances could be of an antibiotic nature and studies of antibiotic production were made by use of penicillin assay pads. Pour plates were made of each of the four organisms, and pads, dipped in sterile filtered medium in which one of the others had grown, were placed on the surface of the inoculated media. Inhibition in each case was produced by a substance that was not removed from the medium by filtering. The inhibitor produced by each of "Unknown B" and *Cylindrosporium* sp. was very active against the other two, but its active principle was removed by heating at

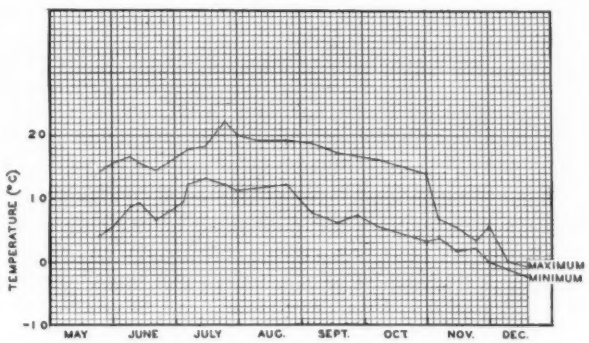


FIG. 5. Maximum and minimum soil temperatures at the top of the B horizon under a yellow birch stand from records taken in 1952.

TABLE III

MYCELIUM PRODUCTION OF FOUR FUNGI WHEN GROWN INDIVIDUALLY IN SEPARATE ALUNDUM THIMBLES IN SHAKE CULTURE, ALONE AND IN ALL COMBINATIONS

		Fungus			
		"Unknown B" (1)	*M. alpina* (2)	*Cephalosporium* sp. (3)	*Cylindrosporium* sp. (4)
Flask No.	Combination	Dry weight (mgm.)			
1	1	63.2	—	—	—
2	2	—	98.7	—	—
3	1,2	59.1	—	—	—
4	3	—	—	22.8	—
5	1,3	57.1	—	—	—
6	2,3	—	72.3	11.1	—
7	1,2,3	64.7	—	0.1	—
8	4	—	—	—	111.2
9	1,4	22.9	—	—	98.8
10	2,4	—	—	—	101.3
11	1,2,4	19.2	—	—	93.9
12	3,4	—	—	0.1	104.6
13	1,3,4	16.2	—	0.2	77.3
14	2,3,4	—	0.1	—	89.6
15	1,2,3,4	27.1	—	0.1	88.3

150° C. for 15 min. When "Unknown B" was grown in association with *Cylindrosporium* sp., one substance produced remained active after heating and it was shown that, when grown together, these two organisms were capable of producing a growth inhibitor that neither could produce alone (21).

Because many observations made in this study indicated the occurrence of competition between root-inhabiting fungi, a test was made between individuals of these four and the fungi more commonly isolated from living yellow birch rootlets to determine the capacity of each to produce a substance or substances that inhibited growth of the others. Tests for such substances were made in two ways. By the first method, all mycelium and spores were removed from a liquid culture by passing it through a Seitz filter, and penicillin assay pads were used as described above. The widths of the bands of inhibition around these pads, when they occurred, were measured to determine the relative strength of an inhibitor against different fungi. Because of the possibility that any growth inhibitor might be absorbed by the cellulose in the Seitz filter or the penicillin assay disk, a second method of testing was used and each pair of fungi was tested in two ways. A spot inoculation of one was made in the center of a pour plate and a narrow ring of inoculum of the second was streaked around it at a distance of about 3 cm. (Fig. 24). The test was repeated by reversing the position of the fungi. Growth of the fungus forming the ring was observed to determine whether rate of growth was different on the outside from that on the inside, and also it was noted if growth of the center one seemed suppressed. The widths of the inhibition zones around the penicillin assay disks are given in Table IV where it is shown

TABLE IV

WIDTH OF INHIBITION ZONE AROUND PENICILLIN ASSAY DISK CONTAINING GROWTH INHIBITOR FROM OTHER FUNGI

Fungus producing inhibitor	Fungus inhibited: Width of inhibition zone (mm.)												
	"Unknown 51-A"	"Unknown 51-C"	"Unknown 51-D"	"Unknown 51-E"	"Unknown 51-F"	"Unknown 51-J"	"Unknown 51-K"	"Unknown 51-L"	"Unknown 51-M"	"Unknown 51-N"	"Unknown B"	*M. alpina*	*Cephalosporium* sp.	*Cylindrosporium* sp.
"Unknown 51-A"	—	—	—	—	—	—	—	1.0	—	—	—	—	—	—
"Unknown 51-C"	2.0	—	—	—	—	—	—	1.0	—	—	—	2.0	—	—
"Unknown 51-D"	—	1.0	—	2.5	—	—	—	—	—	—	—	—	—	—
"Unknown 51-E"	—	1.0	—	—	—	1.0	—	—	—	—	—	—	—	4.0
"Unknown 51-F"	3.0	3.0	1.0	—	—	—	2.0	—	—	—	—	3.0	—	—
"Unknown 51-J"	1.0	1.0	—	—	—	—	3.0	—	4.0	—	—	5.0	—	—
"Unknown 51-K"	—	2.0	2.0	—	—	—	—	—	1.0	1.0	—	—	—	—
"Unknown 51-L"	—	—	—	—	—	4.0	2.0	—	1.5	—	—	2.5	—	—
"Unknown 51-M"	—	—	—	—	—	—	—	—	—	2.5	—	1.0	—	—
"Unknown 51-N"	—	1.0	—	—	2.0	—	—	2.0	—	—	—	—	—	—
"Unknown B"	—	—	—	—	5.0	3.0	3.5	2.5	1.5	4.0	—	4.5	—	—
M. alpina	—	—	—	—	—	—	—	—	—	2.5	—	—	—	—
Cephalosporium sp.	—	—	2.0	—	—	—	—	—	2.0	—	—	1.0	—	—
Cylindrosporium sp.	3.0	4.0	3.0	—	—	2.0	—	2.0	4.0	4.5	2.5	4.5	1.5	—

that no fungus was capable of suppressing growth of all the others and that no fungus was suppressed by all the others. Results of the second test did not reveal the occurrence of any strong inhibition that was not shown by the penicillin assay pad test, but slight suppression of growth not indicated by the first test occurred in several instances. From these results it could be expected that in the soil there is constant competition between fungi and that when temperature, acidity, and other ecological factors are optimum for one fungus or one group of fungi, these can quickly suppress others that are not under optimum conditions.

Effect of Root-inhabiting Fungi on Yellow Birch Seedlings

To determine the effect of the fungi isolated from dead, dying, or mycorrhizal roots of yellow birch on the rootlets of healthy yellow birch plants, a series of inoculations of root systems was conducted on seedlings growing in sterile soil in pots and on agar in flasks. Because loam is much more conducive to copious branching of yellow birch rootlets than sand (20), loam from an optimum site for yellow birch in New Brunswick was used in this and subsequent experiments involving growth of seedlings in pots. It had the following mechanical composition: fine gravel, 1.8%; coarse sand, 6.8%; medium sand, 9.9%; fine sand, 13.9%; very find sand, 11.5%; silt, 33.0%; and clay, 23.1%. Four-and-one-half inch tile flower pots were filled with this soil to within one-half inch of the top; pots and contents were partially sterilized in an autoclave at 102° to 103° C. for 20 min. No soil-inhabiting fungi could be isolated on yeast extract agar following this sterilization. As soon as the soil surface had cooled to 40° C., following sterilization, it was covered with a layer of vaspar (vaseline and parowax in the ratio 1 : 4) about 0.5 mm. thick to prevent contamination of the soil by air-borne fungous spores. When the soil had cooled, a circular piece of vaspar, about 1 to 2 cm. in diameter, was removed from the center of each pot and about 20 seeds, surface sterilized as outlined previously (22), were placed on the freshly exposed soil surface. They were covered by filling the hole with vermiculite, sterilized in dry heat at 140° C. for 90 min. Distilled water, not sterilized but used immediately after distillation, was added daily through the vermiculite in quantity sufficient to keep the soil well moistened. During winter artificial lighting was used to extend the light period to 12 or 13 hr. daily. When the seedlings had grown to a height of 2 to 3 in. and had developed leaves and woody stems, soil inoculations were made as outlined in Table V, five pots with each combination of fungi, which included four isolated from dead and dying roots and 11 most commonly isolated from mycorrhiza. The source of inoculum in each case was a piece of yellow birch root covered with or impregnated by mycelium of the desired fungus (Fig. 25). At the time of inoculation a description was made of foliage development as well as the average height of the seedlings in each pot, and all seedlings were removed from 25 pots for examination of their rootlets to determine the amount of mycorrhizal formation that had occurred through contamination. Since seedlings in only one pot were contaminated, this method of culture was

TABLE V

RESULTS OF SOIL INOCULATIONS ON YELLOW BIRCH SEEDLINGS GROWING IN POTS IN A GREENHOUSE

Fungi used in inoculations	No. of pots	Foliage conditions*		Average mortality (%)	Fungus reisolated	Frequency of mycorrhiza on living rootlets (%)	Root** development	Mantle thickness (microns)	Intracellular hyphae present
		Before inoculation	After inoculation						
B & 51-D	5	C	N	0.2	B	100	VG	5-10	+
B & 51-F	5	N	N	0.2	B	100	G	15-25	+
Cylindrosporium sp. & 51-N	5	N	N	0.4	51-N	100	VG	1-5	+
51-N	5	N	N	1.4	51-N	100	VG	15-20	+
Cylindrosporium sp. & 51-A	5	N	N	2.0	—	100	G	10-15	+
Cylindrosporium sp. & 51-C	5	C	C	2.2	—	100	M	10-15	+
Cylindrosporium sp. & 51-L	5	N	N	2.4	51-L	—	M	—	+
Cylindrosporium sp. & 51-M	5	N	N-C	3.2	—	93	G	10-15	+
Cephalosporium sp. & 51-L	5	N	N	3.2	—	98	VG	1-5	+
Cylindrosporium sp. & 51-D	5	N	C	3.6	51-D	99	—	15-20	+
51-C	5	N	N	3.8	51-C	99	M	5-7	+
Cephalosporium sp. & 51-E	5	N	C	3.8	—	100	VG	10-15	+
Cephalosporium sp. & 51-N	5	N	N	3.8	*Cephalosporium* sp.	100	VG	15-20	+
Cylindrosporium sp. & 51-J	5	N	C	4.0	—	98	G	1-5	+
B & 51-J	5	C	N	4.2	B	100	G	7-12	+
Cephalosporium sp. & 51-D	5	N	N	4.2	*Cephalosporium* sp.	100	G	1-5	+
B & 51-E	5	N	N-C	4.6	B	98	G	1-5	+
B & 51-M	5	N	N	4.6	B	100	G	15-20	+
Cylindrosporium sp. & 51-K	5	N	C	4.8	—	100	F	1-5	+
B	5	C	N	5.0	B	94	M	1-70	+
Cephalosporium sp. & 51-C	5	N	N	5.4	*Cephalosporium* sp.	99	G	15-20	+
Cephalosporium sp. & *M. alpina*	5	C	C	5.6	*M. alpina*	96	M	1-5	+
51-M	5	N	C	5.8	—	100	P	5-10	+
Cephalosporium sp. & 51-A	5	N	C	6.6	—	99	M	1-5	+
Cephalosporium sp. & *Cylindrosporium* sp.	5	N	N	6.8	—	—	G	—	+
Cephalosporium sp. & 51-J	5	N	N	7.4	—	—	G	—	+

* *Foliage:* *N—normal.*
C—chlorotic.
A—absent.

** *Root development:* *VG—very good.*
G—good.
M—medium.
F—fair.

TABLE V—*Concluded*

RESULTS OF SOIL INOCULATIONS ON YELLOW BIRCH SEEDLINGS GROWING IN POTS IN A GREENHOUSE—*Concluded*

Fungi used in inoculations	No. of pots	Foliage conditions*		Average mortality (%)	Fungus reisolated	Frequency of mycorrhiza on living rootlets (%)	Root** development	Mantle thickness (microns)	Intracellular hyphae present
		Before inoculation	After inoculation						
B & 51-K	5	N	C	7.6	B	100	M	15-20	+
Cephalosporium sp. & 51-F	5	C	C	7.6	—	98	M	15-20	+
51-F	5	C	C-A	7.8	51-F	98	G	2-5	−
No fungus	25	N-C	C	8.5	*Penicillium*	12	F	7-10	−
Cylindrosporium sp. & 51-F	5	C	N	8.8	—	96	G	10-15	−
B & 51-N	5	N	C	9.0	B	97	G	20-25	+
Cephalosporium sp. & 51-M	5	N	N	9.2	51-M	99	G	15-20	+
51-J	5	N	N-C	9.4	—	94	M	1-5	+
M. alpina & B	5	N	C	9.4	*M. alpina*	96	M	1-5	+
Cylindrosporium sp. & B	5	C	C	10.0	—	97	G	1-5	−
51-L	5	N	C-A	10.6	—	94	P	1-5	−
B & 51-A	5	N	C	12.6	B	100	VG	1-5	−
M. alpina & *Cylindrosporium* sp.	5	N	C	13.4	—	91	M	1-5	−
B & 51-L	5	N	C	14.6	B	99	M	1-5	−
Cephalosporium sp. & 51-K	5	N	C	15.4	51-K	81	G	15-20	−
B & 51-C	5	C	C-A	15.6	B	98	G	15-20	+
Cephalosporium sp.	5	C	N	15.8	*Cephalosporium* sp.	98	G-VG	1-5	−
51-D	5	N	C	15.8	—	80	F	1-5	−
M. alpina	5	C	C	17.0	*M. alpina*	77	F	1-10	+
Cylindrosporium sp.	5	C	N	17.0	—	—	P	—	
51-A	5	C	C	17.2	—	97	F	1-5	+
Cylindrosporium sp. & 51-E	5	N	C-A	17.6	—	—	P	—	
51-E	5	C	C	18.0	—	—	M	—	
51-K	5	N	C	23.2	—	83	F	1-5	−
Cephalosporium sp. & B	5	N	C-A	34.2	B	95	M	5-10	−

* *Foliage:* *N—normal.*
C—chlorotic.
A—absent.

** *Root development:* *VG—very good.*
G—good.
M—medium.
F—fair.

considered satisfactory, especially in view of the near impossibility of maintaining such a large number of pots (320) under absolutely sterile conditions conducive to plant growth. This study was conducted mainly in the winter to reduce chances of contamination from air-borne spores, inoculations being made in December, 1952.

Seedlings were removed for examination in April and May, 1953, and foliage conditions, development of root systems, frequency of mycorrhizal occurrence, and rootlet mortality were recorded for each pot. Frequency of mycorrhiza was determined by examining a sample of 200 living root tips from each pot and recording the number of mycorrhiza present. Mortality was recorded as the percentage of dead root tips occurring in the first 200 examined. A sample of living rootlets from each pot was sectioned and stained with orseillin BB and crystal violet, as outlined by Cohen and Doak (6), and examined microscopically to determine the average thickness of the hyphal mantle, the occurrence of intracellular and intercellular hyphae, and any other noticeable characteristics. Reisolations were attempted from the rootlets of part of the sample from each pot but the fungi were not always recoverable on the medium used.

The most important result of this experiment is that none of the fungi isolated from yellow birch roots and tested here were capable of virulent pathogenicity against rootlets of yellow birch when grown in pots in a greenhouse. Examination of Table V reveals that mycorrhiza occurred in most cases when the fungi were present. It is noted also that frequency of mycorrhiza in the 25 control plots was 12%. These were found in four pots that were contaminated. Because of contamination, not too much emphasis can be placed on the results listed for occurrence of mycorrhiza, but it is possible that all of these fungi except *Cylindrosporium* sp., *Cephalosporium* sp., and "Unknown 51-E" are capable of forming mycorrhiza with yellow birch rootlets. The average mortality of rootlets listed in Table V was analyzed statistically and differences of 14.3 and 19.1 between averages are statistically significant at the 5 and 1% levels respectively. As to point out all the statistically significant differences would be superfluous, the fungi are arranged in Table V so that mortality increases with descent in the column and observation readily reveals where statistically significant differences exist. However, attention is drawn to the fact that much greater mortality occurred where *Cephalosporium* sp. and "Unknown B" were combined than in any other case. It is suggested that this difference may be due to synergistic interrelations between the fungi that result in production of conditions unfavorable for development of birch roots. Although conditions of the foliage at the time of inoculation and again at the end of the experiment are listed in Table V, no conclusions can be drawn regarding changes in them other than that no fungus was capable of bringing about severe wilting or marked defoliation through any parasitic action or production of toxins.

To test further the capacity of these fungi to act as pathogens, yellow birch seedlings were grown aseptically on agar and inoculated with each of the fungi

listed in Table V. Yellow birch embryos were removed from their seed coats, using sterile techniques described earlier (22), and placed on agar–yeast extract (Fig. 27). As soon as leaves appeared, a drop of spore or mycelium suspension of the fungus to be tested was placed on the medium at a distance of about 1 cm. from the base of the stem. The inoculations were made in duplicate and the flasks were incubated in normal daylight (September) at 16° to 19° C. until results were available. The results showed that, of the fungi tested, none, other than *Cylindrosporium* sp. and "Unknown 51-F", showed any signs of being parasitic against yellow birch seedlings. *Cylindrosporium* sp. cannot be considered a virulent parasite because the stems of seedlings were surrounded by mycelium for three to four days before they were smothered and the leaves remained green until the day before they were covered with mycelium. The other fungus, "Unknown 51-F", caused damping-off but the leaves did not become chlorotic. In accordance with standard techniques for testing parasitism none of these fungi possessed the virulence considered necessary to class them as more than very weak parasites of yellow birch. Under conditions as artificial for growth as those described above the seedlings may be expected to be most vulnerable to a parasitic fungus. Therefore, it seems a certainty that none of the fungi isolated from roots of yellow birch and tested in this experiment can be considered capable of causing birch dieback through parasitic action on the rootlets.

Effect of Strangulation of Root Systems of Yellow Birch on Roots and Crowns

When three-year-old Scotch pine (*Pinus sylvestris* L.) seedlings were strangled with a wire at the base of the stem, mycorrhiza disappeared from their root systems (4). This was considered to be due to the absence of sufficient soluble carbohydrates in the root tips to supply the needs of the mycorrhizal fungi. In view of these results it was considered advisable to strangle a few yellow birch saplings, growing in the forest, and observe the results, not only on the root systems beyond the point of strangulation but also on the foliage of trees completely strangled. Several trees were treated so that only a small portion of their roots lay beyond the point of strangulation while others were strangled so that the total system was deprived of all downward transmission through the phloem of materials from the crown (Fig. 28). To bring about strangulation, heavy wire was twisted tightly until it compressed the phloem completely around the root or stem. All treatment was applied in May at the time when foliage buds were bursting and observations were begun 8 weeks later and continued periodically for 16 months. Examination of rootlets eight weeks after treatment showed that except on larger roots (over 4 cm. in diameter at the point of strangulation) all mycorrhiza beyond the point of strangulation had withered up and appeared dead. In one case where one branch of a dichotomously branched root was strangled two inches beyond the crutch and the other was undisturbed, all rootlets distal of the point of strangulation were dead and the mycorrhiza had

collapsed and were dull in color (Fig. 29), whereas those on the other branch were healthy and the mycorrhiza were fleshy and lustrous (Fig. 30). In this case, the rootlets from each branch were growing intermingled in the same soil horizon. Two trees that were strangled a few inches above the ground (Fig. 28) were observed in August to have produced shorter internodes than untreated trees in the immediate vicinity. Twelve weeks after treatment the entire foliage of these trees was chlorotic, all their rootlets were dead, and the mycorrhiza had collapsed. These trees failed to produce foliage the following season. It is considered that the most important result of this experiment is that destruction of portions of the rootlets, through strangulation, brought about symptoms similar to those of birch dieback.

Effect of Soil Temperature on Development of Yellow Birch

Because of the lack of temperature control and the great chance of contamination in the previously described study of seedlings grown in the greenhouse, another study was initiated in a specially constructed chamber in a room where periodic spraying with a fungicide was employed to diminish chances of contamination by air-borne spores. The object of this study was to determine more closely the effect of a selected few of the fungi on root systems of yellow birch and simultaneously to observe differences in root development, mortality, and occurrence of mycorrhiza at different temperatures.

A chamber was constructed, with a heating element at the bottom of one end and a refrigeration unit at the top of the other (Fig. 6), of 2-in.-thick spruce; the inside and outside walls were of insulboard; and space between the walls was filled with vermiculite. No insulation was used on the floors which were of thick concrete. Interior dimensions of the chamber were 96 in. long × 42 in. wide × 36 in. high. A sheet of galvanized metal 27 in. wide, which provided space for six rows of nine $4\frac{1}{2}$-in. flower pots, was arranged horizontally at about mid-height between the heating and refrigeration units and the ends were bent down and up respectively to contact the units. To

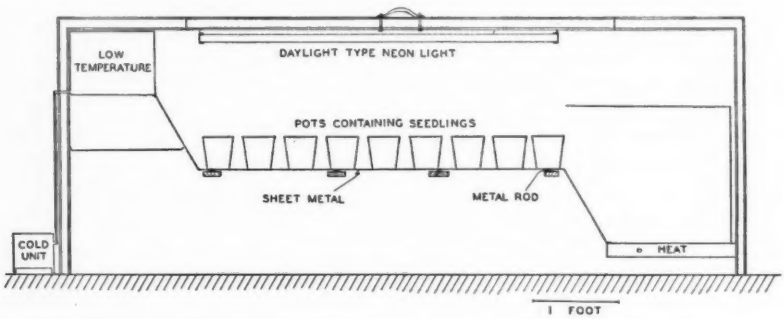


FIG. 6. Diagram of chamber used to obtain a range of temperatures, constant at any one position.

promote rapid growth, the whole chamber was lighted continuously with daylight-type neon bulbs. The refrigeration unit was operated continuously, while the heating unit was controlled by a thermostat regulated to keep the range of temperatures in the pots at a desired level. To obtain a reasonable estimate of the optimum temperature for development of yellow birch, a maximum–minimum thermometer was buried in the soil under a yellow birch stand at the depth of maximum rootlet development. This thermometer was read and reset periodically from May 1 to December 1, 1952. It was considered that the optimum temperature range would occur between July 1 and September 15, the period when seedling establishment occurs. The highest maximum and minimum temperatures recorded during this period are shown in Fig. 5 to be 13° and 22° C. respectively, and a range of 16° to 21° C. was selected for use in the chamber. Measurement of temperatures showed that all pots equidistant from either unit were at the same temperature level, whereas there was a difference of approximately 0.5° C. between the temperatures of soil in adjacent pots equidistant from the heating or cooling unit. The temperatures in the centers of successive pots, reading from cooling unit to heating unit, were adjusted to remain constant at 16.4°, 17.0°, 17.6°, 18.1°, 18.7°, 19.2°, 19.8°, 20.3°, and 20.9° C., respectively.

Soil used in this experiment was obtained from the same source as that used in the pots where seedlings were grown in the greenhouse, but the layer of vaspar was not used; rather, a 0.5-in. thick layer of sterilized vermiculite was placed over the seeds after they had been surface sterilized and placed on the surface of the sterilized soil. The pots were watered daily with enough sterile distilled water to keep the soil moist, care being taken to avoid water seepage from the bottom of the pots. Seeds were planted January 6, 1953, and on April 7, 1953, when the seedlings were from one to four inches tall, five rows of nine pots each were inoculated with the following fungi or combinations of fungi: "Unknown B", *Cylindrosporium* sp., "Unknown 51-N", *Cylindrosporium* sp. and "Unknown B", and *Cylindrosporium* sp. and "Unknown 51-N". One row of pots was inoculated with sterile pieces of birch root and served as a check. On June 6, 1953, the plants were removed from the pots and rootlet development, rootlet mortality, and frequency of mycorrhiza were determined. A sample of rootlets was selected from three pots in each of the six rows for microscopic examination of the type of mycorrhiza, when present, and any other characteristics that could be related to different temperatures. These pots were at the 16.4°, 18.7°, and 20.9° C. positions.

Yellow birch seeds for this study could not be obtained from the Maritime Provinces so it was necessary to use a collection from Dorset, Ontario, the nearest available source. From Fig. 31 and Table VI it would appear that the optimum temperature for development of yellow birch from this source lies between 17.5° and 19.5° C., possibly close to 18.5° C. Rootlet mortality was lowest and development of foliage was best in this range. Development of mycorrhiza also was best in this range, or in a slightly larger range

TABLE VI

ROOTLET MORTALITY, FREQUENCY OF MYCORRHIZA, AND THICKNESS OF THE HYPHAL SHEATH OF MYCORRHIZA IN RELATION TO SOIL TEMPERATURE IN POTS

Fungus	Temperature (° C.)									
	16.4	17.0	17.6	18.1	18.7	19.2	19.8	20.3	20.9	Av.
Rootlet mortality (%)										
None	90	32	5	3	6	3	9	12	31	22.3
Cylindrosporium sp.	75	48	21	13	12	11	65	46	28	34.3
"Unknown B"	100	30	1	1	1	2	3	9	20	18.6
Cylindrosporium sp. & "B"	1	2	0	0	2	2	4	4	10	2.8
"Unknown 51-N"	20	1	0	0	1	1	2	10	9	4.9
Cylindrosporium sp. & "51-N"	33	39	28	55	64	65	70	78	81	56 4
Average	53.3	33.3	9.2	9.1	9.6	9.3	25.5	26.3	29.8	
Frequency of mycorrhiza (% of living rootlets)										
None	0	0	0	0	0	0	0	0	0	0
Cylindrosporium sp	0	0	0	0	0	0	0	0	0	0
"Unknown B"	—	93	99	100	100	100	100	64	33	86.1
Cylindrosporium sp. & "B"	90	85	80	90	99	92	80	50	31	77.4
"Unknown 51-N"	70	100	100	100	100	100	100	100	100	96.7
Cylindrosporium sp. & "51-N"	80	78	100	100	100	100	100	100	90	94.2
Average	80.0	89.0	94.8	97.5	99.8	98.0	95.0	78.5	63.5	
Thickness of hyphal sheath on mycorrhiza (microns)										
None		—			—			—		
Cylindrosporium sp.		—			—			—		
"Unknown B"		—			15-20			1- 5		
Cylindrosporium sp. & "B"		10-15			15-20			5-10		
"Unknown 51-N"		1- 5			15-20			10-15		
Cylindrosporium sp. & "51-N"		1- 5			15-20			5-10		

encompassing the same mean optimum temperature. More specifically, examination of Fig. 31 shows seedlings grown at 18.7° C. were most vigorous, and had, on the average, the best foliage development and survival. Those at the extremes of the range were still in the cotyledon stage, if still alive, and had spindly stems. Again it is shown that *Cylindrosporium* sp. was not capable of forming mycorrhiza with yellow birch and also it is apparent that frequency of mycorrhiza was reduced slightly when *Cylindrosporium* sp. occurred in the soil with either of the two fungi, "Unknown B" or "Unknown 51-N", which were capable of forming mycorrhiza. This is especially noticeable when *Cylindrosporium* sp. and "Unknown 51-N" were combined. Also, when it is considered that at 20.9° C., when these two fungi were combined, only 19% of the rootlets remained alive and only 90% of these had mycorrhiza, it is apparent that there was little formation of mycorrhiza (17% of all rootlets). In Table VII where the frequency of mycorrhiza is given as a percentage of all rootlets, living and dead, it appears that there may have been some interaction between *Cylindrosporium* sp. and "Unknown B" that caused increases in frequency of mycorrhiza at lower temperatures and decreases at higher temperatures, when compared with their frequency when "Unknown B" occurred alone. According to Table VI the thickness of the hyphal sheath was greatest in all cases where mycorrhiza occurred when the optimum temperature prevailed. At the extreme high and low temperatures it was much thinner. "Unknowns B and 51-N" were reisolated from the

TABLE VII

FREQUENCY OF MYCORRHIZA AS A PERCENTAGE OF ALL ROOTS OF YELLOW BIRCH SEEDLINGS GROWN IN POTS AT DIFFERENT TEMPERATURES

Fungus	Temperature (° C.)								
	16.4	17.0	17.6	18.1	18.7	19.2	19.8	20.3	20.9
None	0	0	0	0	0	0	0	0	0
Cylindrosporium sp.	0	0	0	0	0	0	0	0	0
"Unknown B"	0	65	98	99	99	98	97	58	27
Cylindrosporium sp. & "B"	89	83	80	90	97	90	77	48	28
"Unknown 51-N"	56	99	100	100	99	99	98	90	91
Cylindrosporium sp. & "51-N"	54	48	72	45	36	35	30	22	17

roots in pots that had been inoculated with them but *Cylindrosporium* sp. was not reisolated. *Penicillium* sp. was isolated from dead roots in one pot inoculated with *Cylindrosporium* sp. and from one of the check pots. Therefore, it is assumed that soil in these pots remained free of contaminants capable of forming mycorrhiza on yellow birch, none being isolated on the medium used.

These results show that soil temperature can influence the balance of microbiological populations, the development of mycorrhiza, and the vigor of rootlets of yellow birch. To investigate this further, soil in a restricted area supporting yellow birch trees was artificially heated by known amounts and the effects on yellow birch rootlets were measured. A 20 ft. × 30 ft. plot was selected in southern New Brunswick on a moist slope with a northerly exposure that contained about one-half acre of a hardwood mixture, 45 to 55 years old, of which yellow birch comprised more than 50% of the stand. This stand originated following a fire about 1895 and since that time had suffered no interference through cutting, burning, or windthrow, and none of the yellow birch trees had suffered deterioration that could be attributed to dieback. The position of trees on the plot is shown in Fig. 7. A soil profile and the relative occurrence of yellow birch feeding rootlets in each horizon are described in Fig. 8. A mechanical analysis of soil taken from the top of the B horizon at four locations on the plot showed the soil to be a clay loam. Heat was applied to the soil by means of a common type of greenhouse soil-heating electric cable, buried at a depth of 4 to 5 in.—in the bottom half of the B horizon—in a narrow trench made by careful removal of the soil by hand. Cables were threaded through and below the roots in such a manner that only a minimum number of rootlets was destroyed or damaged. Soil was then packed around the cables to the original level.

Soil temperatures were measured by using a potentiometer connected to thermocouples, made of copper–constantan 24-gauge wire with polyvinyl plastic coating, arranged in the soil in four parallel lines, 19 per line, spaced seven feet apart, and running perpendicular to the direction of the cable (Fig. 9). They were placed during a cloudy day following a heavy rain at a depth of about two inches in the mineral soil, the top of the B horizon, where

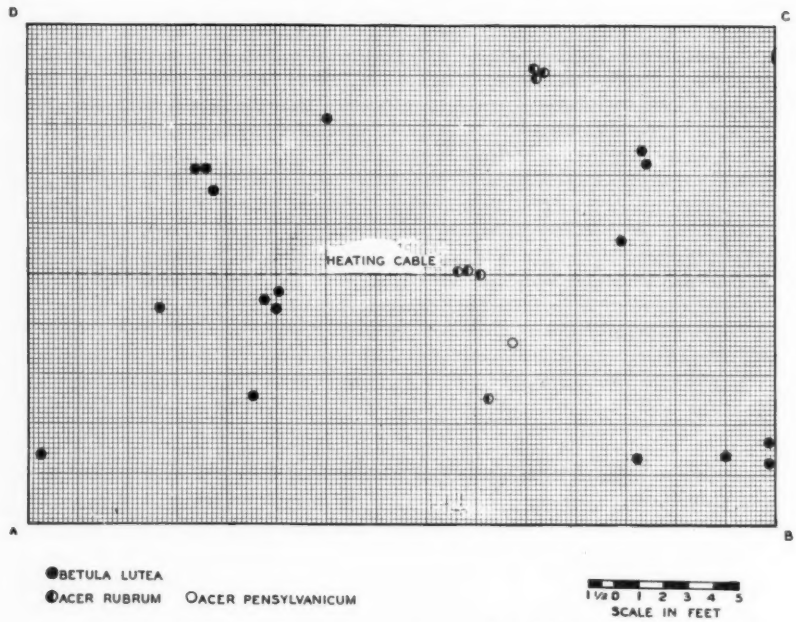


FIG. 7. Location of trees by species on the experimental plot used in soil heating experiments.

the majority of the feeding rootlets of yellow birch were located (Fig. 8), the temperature at each was determined, the average temperature of all was calculated and each not at the average was then lowered or raised in the soil until it was in an area with a temperature equal to the average of the first readings. The thermocouples in each line were placed one foot apart at the beginning (Fig. 9), but were later relocated so that the 19 thermocouples were at 4-in. intervals in each line. To facilitate reading so many, those in each line were connected to a dial as shown in Fig. 10 and in Fig. 32, and all in each line were placed in series with one that was inserted in the center of a thermos containing ice (0° C.). Thus, the reading recorded for any one thermocouple was the difference in resistance between that one and the one in the thermos and was readily converted to °C., the temperature at the location of that thermocouple in the soil. Additional thermocouples were placed at the soil surface in the shade to determine air temperature at the surface at the time that soil temperatures were measured. These results are presented graphically in Fig. 11. Soil moisture as a percentage of oven-dry weight was measured by means of Colman soil moisture units placed, at the same depth as the thermocouples, six inches apart in two parallel lines perpendicular to the direction of the heating cable (Fig. 9). Temperature and moisture measurements were made twice daily, between 8.00 and 10.00 a.m.,

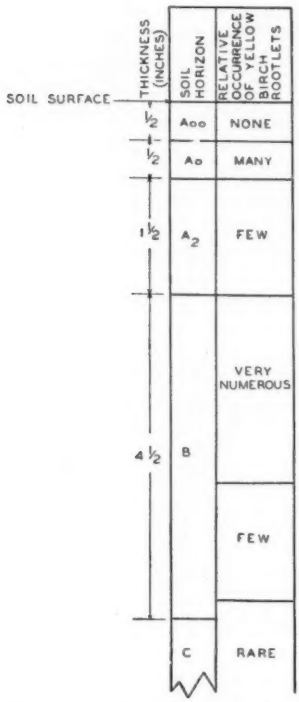


FIG. 8. Soil profile from the experimental plot where soil heating was conducted. The relative occurrence of yellow birch rootlets in each horizon is indicated.

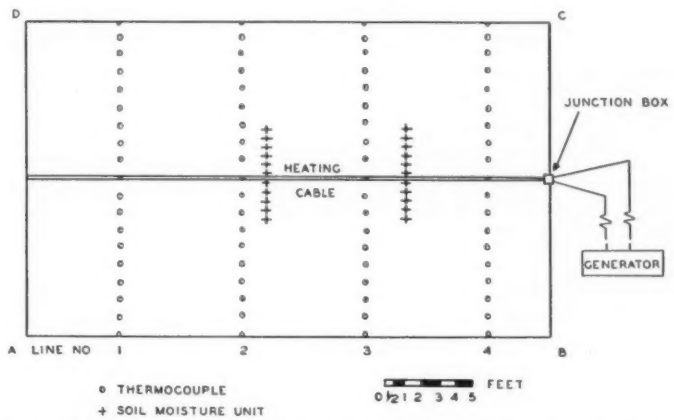


FIG. 9. Diagram of the experimental plot showing the location of the heating cable, thermocouples, and soil moisture units.

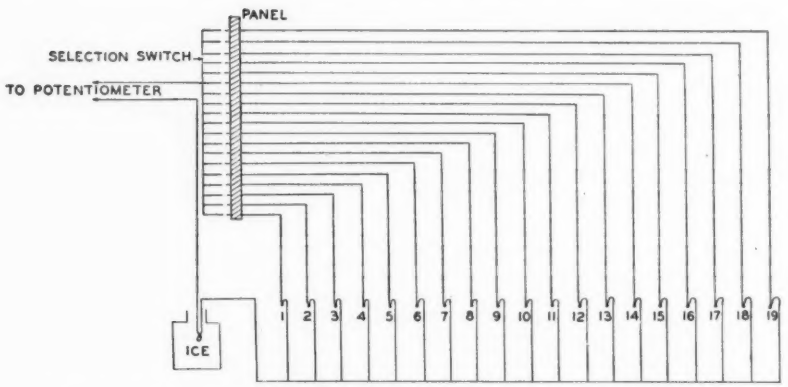


FIG. 10. Diagram of the wiring of the 19 thermocouples in each line at the experimental plot as inserted in the soil and arranged in series with a 20th inserted in crushed ice.

and between 12.30 and 2.30 p.m. (local standard time), when it was not raining during those periods. During periods of precipitation, inaccuracies arose through moisture causing electrical disturbances in the recording instruments, but it was not considered advisable to build a shelter over the instruments because it was feared that one large enough to be effective would interfere with climatic factors affecting the plot, and thereby introduce uncontrollable errors.

Heating was begun June 3, 1953, and applied continually until September 6, 1953. Temperature recordings were made throughout this period but soil moisture was not recorded until August 16. Samples of roots, collected September 6 and October 14, were excavated by hand and the exact distance of their origin from the heating cable was recorded. Records were made of mortality of rootlets, frequency of mycorrhiza on living rootlets, and development of new rootlets. Fig. 13 shows the increases from the normal soil temperature that occurred each day at various distances from the cable. Precipitation data (Fig. 13) and maximum and minimum air temperatures (Fig. 12) were obtained from the Acadia Forest Station Headquarters, three miles from the experimental plot, the nearest open area where such measurements could be made. Because of irregularities in the curves in Fig. 13 an attempt is made in Fig. 14 to smooth the graphs by using the system of moving averages and at the same time to learn how nearly parallel they may be. The curve in Fig 15 is plotted to show the relation between the average increase in soil temperature throughout the summer and distance from the cable. The data obtained from observation of rootlet mortality are plotted as mortality over distance from the cable in Fig. 16. The data from Fig. 15, increase in soil temperature plotted over distance from the cable, are combined with the information from Fig. 16, rootlet mortality in yellow birch in relation to distance from the cable, and a graph is constructed to show the relationship

between rootlet mortality and increases in soil temperature above that normal for the season (Fig. 17). This shows that rootlet mortality in yellow birch was increased from an average of about 6% to 19% by an increase of 1° C. in the soil temperature throughout the summer, an increase of 2° C. raised mortality to near 60%, and a 6° to 7° C. increase resulted in death of all rootlets. Excavations made October 14 showed that new rootlets had begun already to develop in the soil where killing had occurred earlier because of heating. On September 6, new rootlet development was found at a depth of six inches under an area where all roots two to three inches deep had been killed. Obviously, the tree was attempting adjustment to the altered environment.

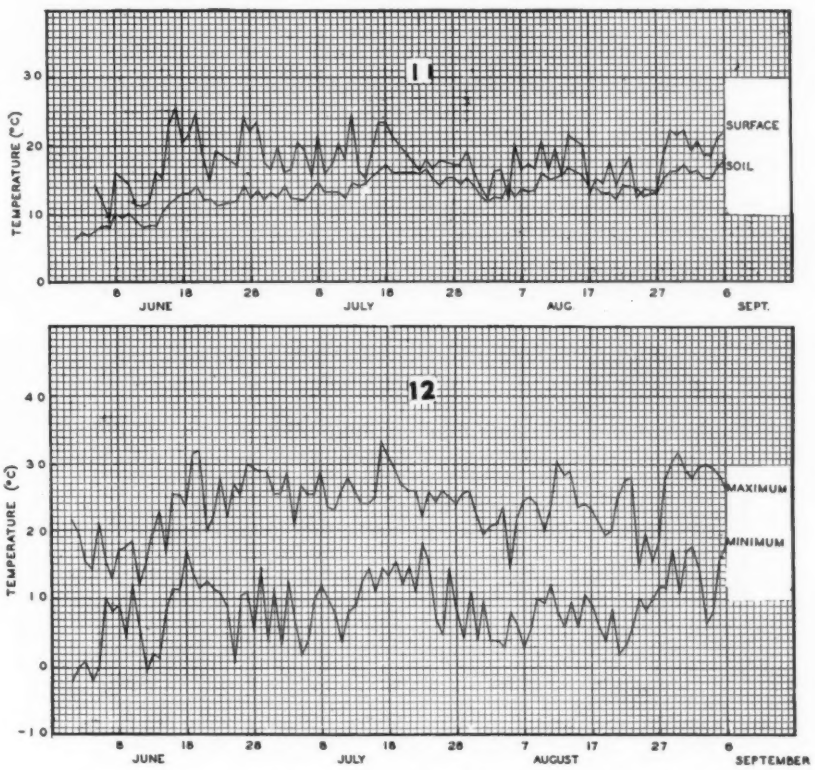


FIG. 11. Temperatures at the upper surface of the soil, upper curve, and those at the depth of maximum development of yellow birch rootlets, lower curve, as recorded daily from June 5 to September 6, 1953.

FIG. 12. Maximum and minimum air temperatures recorded during the period June 3 to September 6, 1953, the period when soil heating was conducted.

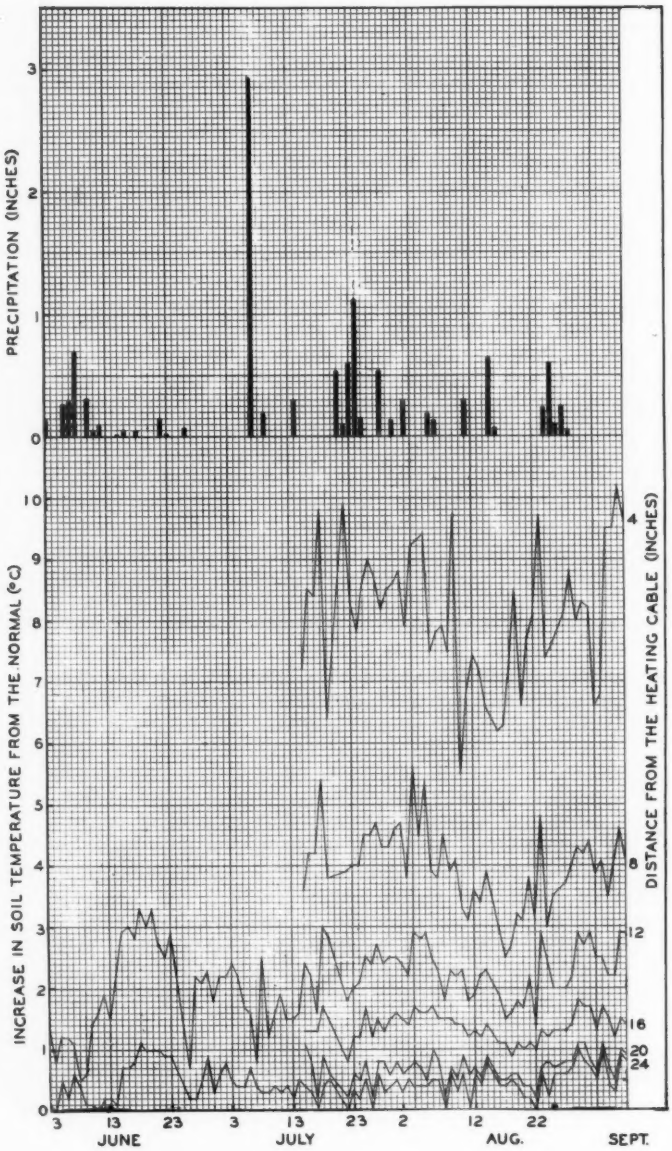


FIG. 13. Increases in soil temperature from the normal as related to distance from the heating cable, measured daily from June 3 to September 6, 1953. Precipitation in inches is also included.

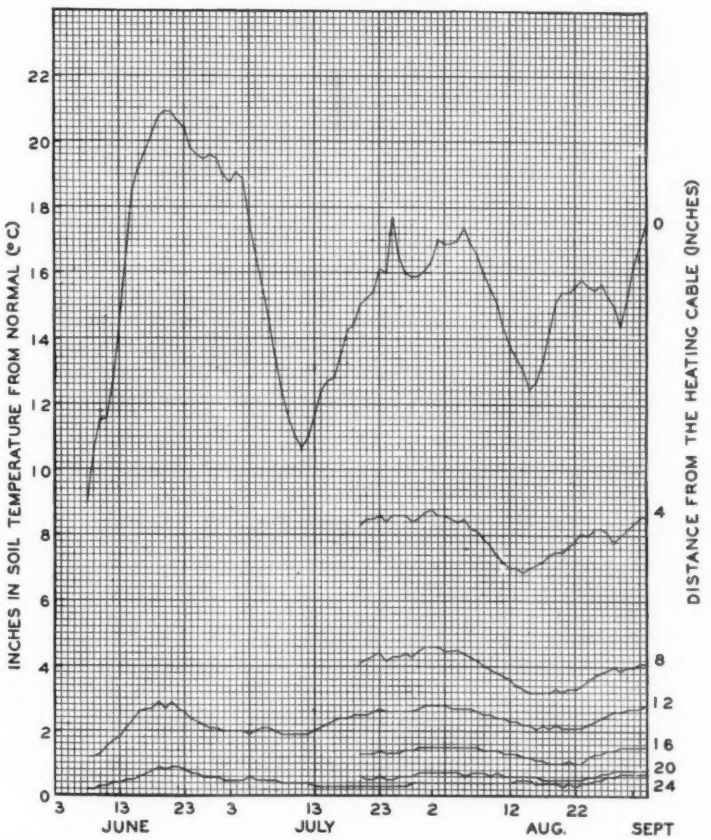


FIG. 14. Moving averages of actual soil temperatures at depth of maximum rootlet development from June 8 to September 2, 1953. Temperature at any point is the average of that recorded for that day, the preceding five, and the succeeding four days.

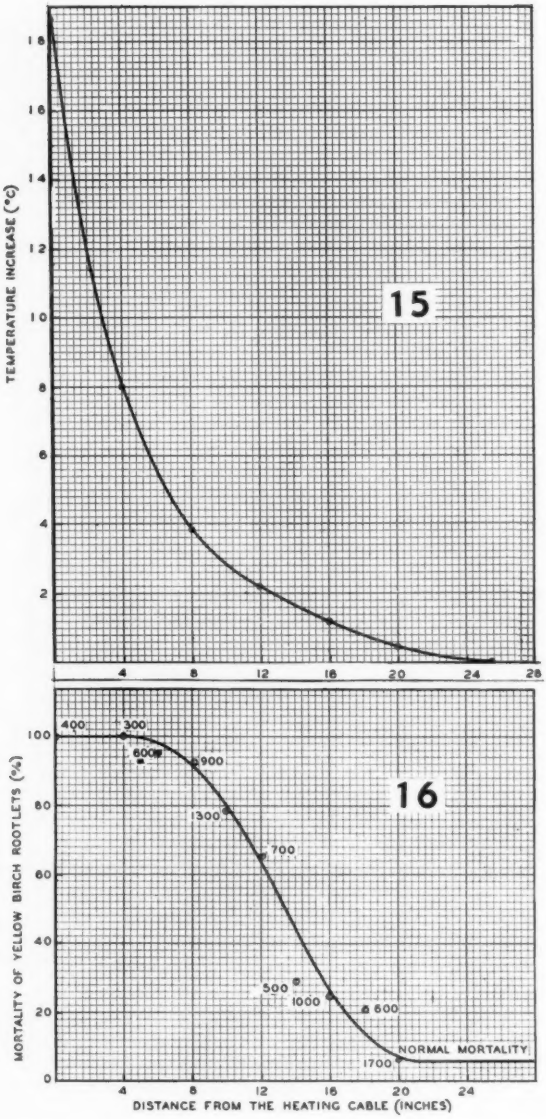


FIG. 15. Average increase in soil temperature in relation to distance from the cable, computed for the duration of soil heating.

FIG. 16. Mortality in yellow birch rootlets in relation to distance from the heating cable, three months after heating was initiated.

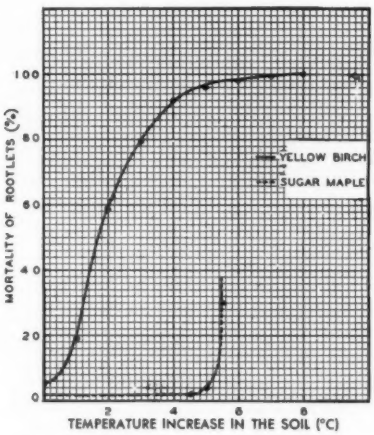


FIG. 17. Relation between mortality in yellow birch and sugar maple rootlets and increase in soil temperatures throughout the growing season.

Sampling of mycorrhiza revealed that there was no significant decrease in their frequency of occurrence on rootlets that had survived increased soil temperatures. However, in areas where mortality was in the vicinity of 40 to 60% the moniliform type of structure illustrated in Fig. 26 was found frequently. The mycorrhizal structures on rootlets killed by increased temperatures were shrivelled and no fungus except soil molds could be isolated from them.

Fig. 17 shows also that sugar maple rootlets are not so susceptible to small temperature changes and increased mortality was not brought about until the soil temperature was raised approximately 5° C. Because only a few rootlets from one sugar maple tree occupied the area where soil temperature was increased, the results can be considered only as indicative of relative differences between the two species in regard to susceptibility to increased soil temperatures. The average mortality normally occurring in sugar maple rootlets is much less than that in yellow birch, being about 1 or 2%.

In Fig. 18 the relationship is given between soil moisture content and the duration of increased soil temperature following periods of precipitation. The curve designated normal soil temperature is actually the average of temperature readings at 12, 18, 24, and 30 in. from the cable, the distances at which the data to obtain the curve for normal moisture content were obtained. From Table VIII it is apparent that there are statistically significant differences between the moisture content at various points in the soil, regardless of their distance from the heating cable, and also that the moisture content for line 1 is highly significantly greater than that for line 2. No explanation can be given for these differences except the heterogeneous condition of the soil.

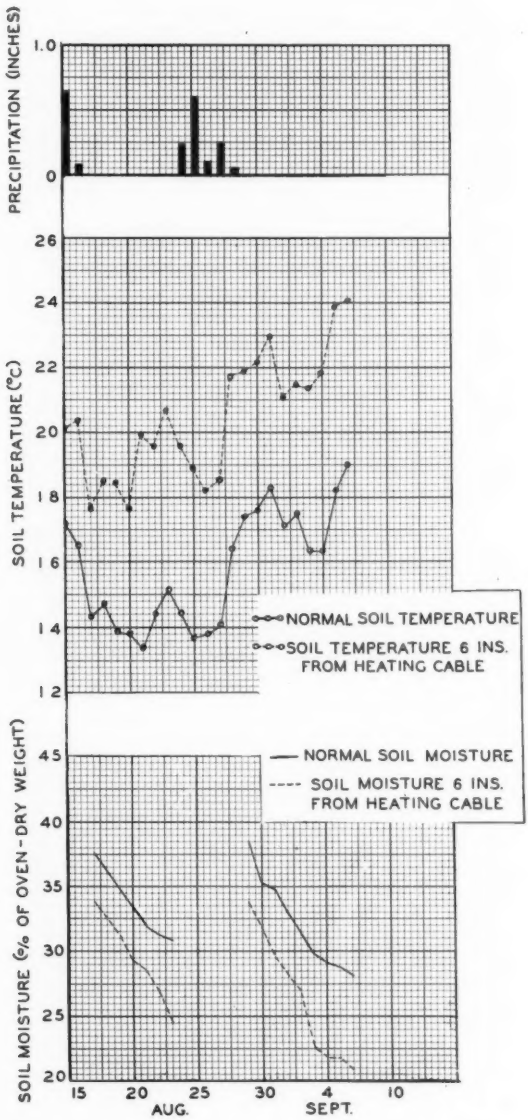


FIG. 18. Normal moisture content of the soil and that at six inches from the heating cable in relation to soil temperature in those positions. Precipitation is also shown.

TABLE VIII

AVERAGE READINGS OF SOIL MOISTURE UNITS AS INDIVIDUALS, IN LINES, AND BY DISTANCE FROM HEATING CABLE, AUGUST 16 TO SEPTEMBER 6

Distance from cable (in.)	Line 1		Line 2	
	Serial No. of soil moisture unit	Av. of 38 readings (soil moisture as % O.D. wt.)	Serial No. of soil moisture unit	Av. of 38 readings (soil moisture as % O.D. wt.)
6	1905	27.9	1915	25.8
	1906	34.9	1916	22.4
12	1904	36.8	1914	26.4
	1907	34.4	1917	26.9
18	1903	34.2	1913	38.1
	1908	32.8	1918	34.6
24	1902	32.9	1912	28.6
	1909	30.0	1919	33.3
30	1901	38.3	1911	30.6
	1910	33.6	1920	31.2
Average		33.6		29.9

Just significant differences:

	1% level	5% level
Between units	3.8	2.9
Between lines	1.4	

TABLE IX

AVERAGE SOIL MOISTURE CONTENT (O.D. WT. BASIS) IN RELATION TO DISTANCE FROM HEATING CABLE AND LENGTH OF TIME SINCE RAIN

Days since rain	No. measurements in each average	Distance from heating cable (in.)				
		6	12	18	24	30
1	8	33.8	36.3	41.3	36.3	38.5
2	8	32.1	33.5	38.1	34.0	34.5
3	8	29.3	32.9	37.6	32.9	35.6
4	8	28.8	31.6	35.0	31.8	34.3
5	8	27.0	29.4	33.9	30.3	32.9
6	8	24.8	30.0	32.9	29.2	31.5
7	8	23.1	29.5	31.0	28.5	31.4
8	4	21.8	28.5	30.3	27.5	29.5
9	4	21.0	27.5	29.3	28.8	28.0
Average	64	27.6	31.4	34.9	31.4	33.4

Just significant differences:

	1% level	5% level
$N = 4$	7.9	6.0
$= 8$	5.6	4.2
$= 20$	3.5	2.7
$= 40$	2.5	1.9
$= 64$	1.4	1.1

In Table IX measurements taken immediately following rainy periods are omitted and it is shown that the average soil moisture content six inches from the cable was highly significantly less than at twelve inches or at any farther point. Also, when daily measurements at different distances from the cable are examined and the just significant differences at the bottom of Table IX are applied, it is shown that by the sixth day the moisture content is less ($P < .05$) at 6 in. than at 12 in. or farther, and that by the seventh day significance reaches the 1% level. On the eighth and ninth days the differences are not statistically significant at the 1% level because measurements were taken on one day only in each case, but the trend is obviously significant (Fig. 18).

In summation it can be stated that an increase in soil temperature of 5° C. above the normal did not lower the soil moisture content a statistically significant amount below that resulting from normal water loss until about the sixth day after the soil had been at field capacity. At no time did the moisture content reach the wilting coefficient, calculated to be 13% for this soil. It is shown conclusively that a small increase in soil temperature above that normally prevailing can bring about rootlet mortality much greater than that normally occurring in yellow birch. In this case, initiation of the increase was abrupt and the roots had little chance to prepare defense against it through relocation as would have been possible if it had been brought about gradually, and it is therefore possible that the results may not be the same as if the increase had been brought about gradually over a period of years. In considering the effect of increased temperature on moisture content, it seems safe to assume that the deleterious effect on the rootlets brought about by soil heating was mainly due to increase in soil temperature and only slightly, if at all, because of the indirect effect of drying caused by the temperature increase. To explain why the moisture content was not lowered more rapidly in the heated areas the manner in which moisture escapes from soil must be considered. Presumably the moisture content of this soil during the first days after a rain was sufficient to permit free movement of water throughout, and even after several days of dry weather it is unlikely that the relative humidity of the soil atmosphere was much less than 100%. It has been shown (10) that any water moving in the soil atmosphere would be towards colder and wetter soil or, in this case, downward and away from the cable. Water loss by means of surface evaporation would have been limited because of the mulching effect of the litter. Another way that moisture is removed normally from soil supporting trees is through absorption by living rootlets of plants. When it is recalled (Fig. 16) that between 90 and 100% of the yellow birch rootlets six inches from the cable had been killed, and that rootlets of this species comprised the great majority of those in the soil at that distance, it is evident that water loss from this source was almost negligible. This indicates that any small increase in soil temperature that kills tree rootlets reduces the source of removal of much soil moisture and, until foliage is removed to permit greater insolation, the rate of water loss may not be greatly increased—probably not at all.

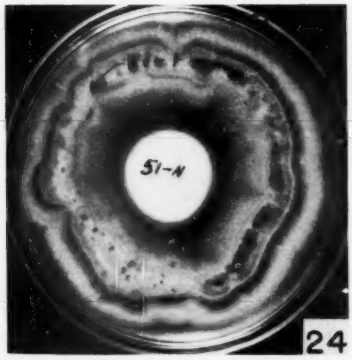
FIG. 19. Mixed wood stand in Nova Scotia. All yellow birch have been killed by dieback.

FIG. 20. Yellow birch tree with normal healthy foliage.

FIG. 21. Yellow birch tree with bare twigs and thin foliage in periphery of crown.

FIG. 22. Yellow birch tree with sparse bunched foliage and dead branches.

FIG. 23. Yellow birch tree with over half the crown dead. Improvement is shown by the normal foliage arising from surviving or adventitious branches at the base of the crown.



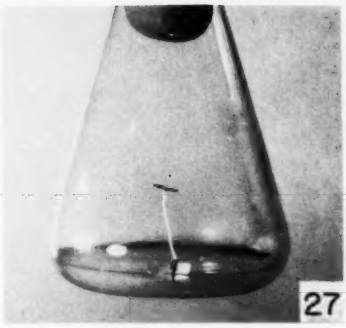


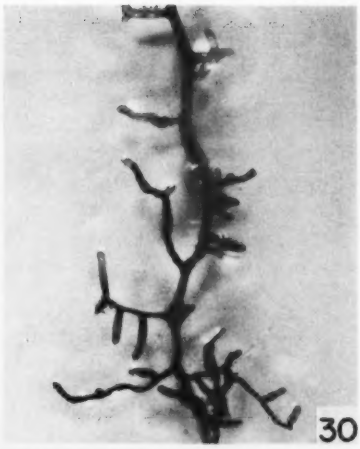
FIG. 24. "Unknown 51-N" growing in center and "Unknown 51-F" on the outside. "51-N" has suppressed inward growth of "51-F" slightly.

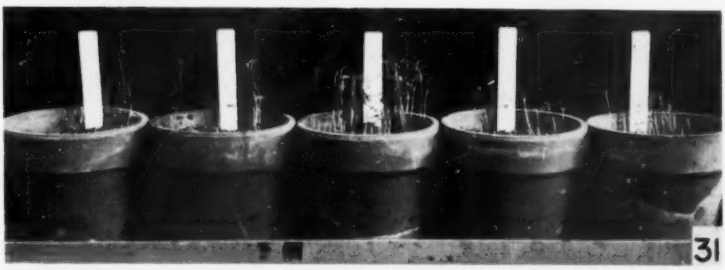
FIG. 25. Flask containing sections of yellow birch roots inoculated with *Epicoccum purpurascens*.

FIG. 26. Moniliform mycorrhiza from yellow birch. Commonly found during winter and occasionally when increased soil temperature in summer is near the limit for rootlet survival.

FIG. 27. Yellow birch seedling growing aseptically on an agar medium.

FIG. 28. Yellow birch sapling strangled with heavy wire at a height of about eight inches.





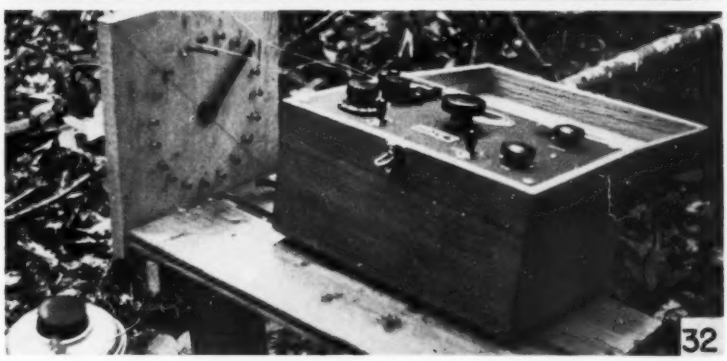


FIG. 29. Collapsed mycorrhiza of yellow birch. This condition was brought about by strangling the root system with wire.

FIG. 30. Fleshy, lustrous mycorrhiza of yellow birch. Note radiating hyphae from root tips.

FIG. 31. Yellow birch seedlings three months old, grown in sterile soil under continuous light and constant temperature. The temperature at each pot from left to right was 16.4°, 17.6°, 18.7°, 19.8°, and 20.9° C. respectively.

FIG. 32. Panel for one line of thermocouples used in measuring soil temperatures. The potentiometer and thermos of crushed ice are in place for taking measurements.

An increase of 1° to 2° C. is reported (14) to have occurred in the air temperatures of the Maritime Provinces during recent years and it is considered that this would have caused an increase in soil temperatures. Fig. 11 shows that air temperatures do affect soil temperatures. For instance, on June 8, the air temperature was 7° C. higher than on the previous day; the soil temperature increased 2° C. Similarly, a drop of 6° C. in air temperature from June 20 to June 21 resulted in a drop of nearly 2° C. in soil temperature. These effects were registered in less than 24 hr. and certainly would have been greater with longer duration of alterations. It is noted that there is a time lag involved in the relative fluctuations and it is shown also that three times during August the surface temperature dropped below the soil temperature.

Discussion

No organism that can be considered responsible for the widespread disturbance known as birch dieback has been found attacking aerial portions of birch trees, and results presented here show no root-inhabiting fungus to be present consistently enough or to have the pathogenicity required to cause the rootlet mortality found in trees deteriorating rapidly. There is a possibility that a virus yet to be discovered is responsible. Some authors (2, 13, 14) have suggested that changes in climate or environmental changes resulting from man-made disturbances could have been the cause and it has been reported (14) that there has been an increase in the average summer temperature in Nova Scotia and New Brunswick of from 1.0° to 1.4° C. during a 30-year period prior to 1950. Because rootlet mortality was reported to be the first symptom of dieback (15) it was considered that any temperature changes great enough to cause this disturbance would act through the soil. It is shown here that temperature increases upset the population balances of fungi inhabiting roots and the rhizosphere and disturb the development of mycorrhiza. Moreover, small increases in soil temperature above those normally occurring in the soil result in greatly increased mortality of rootlets in yellow birch; an increase of 2° C. after 100 days resulted in an increase from normal mortality of about 6% to about 60%, and an increase of 7° C. resulted in 100% mortality. It is therefore probable that higher summer temperatures sufficient to raise soil temperatures 2° C. could cause rootlet mortality sufficient to result in a condition resembling birch dieback. In this study heating was not applied to a sufficient amount of any root system to cause decline of vigor in foliage. However, it is difficult to reconcile the reported spread of dieback from a focal point in New Brunswick (3, 18) with a warmer climate unless higher temperatures originated and spread from approximately the same point. There is no satisfactory evidence that this is so.

In considering the silvicultural treatment of birch stands or mixed stands of yellow birch and sugar maple, the main problem is that of cutting in such a way that residual birch will survive. Results of this study show that yellow birch rootlets are seriously affected by small increases in soil temperature.

Thus, it is certain that yellow birch will not survive a severe stand disturbance. Future management plans and silvicultural treatments of northeastern hardwood forests containing yellow birch should definitely not permit increases in soil temperatures that could affect residual yellow birch trees adversely, if deterioration is to be avoided. Furthermore, in pure birch stands, if clear cutting is not practised, any stand disturbances should be avoided.

Acknowledgments

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NUCLEAR DIFFERENCES IN *ALLIUM CEPA* ROOT TISSUES AS REVEALED THROUGH INDUCTION OF MITOSIS WITH INDOLEACETIC ACID

By A. L. Chouinard[2]

Abstract

Treatment with the growth substance indoleacetic acid was used to stimulate mitosis in differentiated tissues of *Allium cepa* roots in order to establish whether nuclear differences, specifically in chromosome number, exist normally and are merely revealed by treatments, or whether these nuclear differences are produced by the treatment. With the concentrations of indoleacetic acid and durations of treatment used, evidence was obtained that chromosome number differences exist in the differentiated tissues of onion root prior to any treatment and that the appearance of these differences after treatment is a matter of revelation and not of causation. The evidence obtained in these investigations comes indirectly from observations of nuclear size and structure and directly from rare spontaneous mitoses in untreated roots, and in the treated roots (i) from the absence of nuclear abnormalities which might explain otherwise the origin of polyploid cells, (ii) from the uniform occurrence of certain chromosome numbers in specific root tissues, (iii) from proportions of diploid and polyploid divisions during mitotic induction, and (iv) from the lack of relationship between total mitotic activity and number of polyploid mitoses. A gradient of endomitotic polyploidization exits in certain tissues along the longitudinal axis of the onion root.

Introduction

It has been generally assumed that the various tissues in a higher organism retain the original gene quantity of the nucleus of the fertilized egg. In recent years, however, Geitler (9, 10) and Huskins (12) have shown that cell and tissue differentiation sometimes are accompanied by visible changes in the nucleus and that, at least in a quantitative way, all cells in an organism are not genetically alike.

Through observation of spontaneous mitoses in differentiated tissues, the simultaneous presence of diploid and polyploid cells within one tissue (polysomaty) has been reported for many species of plants and animals. The classical investigations of Gentcheff and Gustafsson (11) on *Spinacia* and of Geitler (8) on *Gerris* have considered that the polyploid condition of certain somatic cells arises as a result of repeated chromonenal reproduction within an intact nuclear membrane. This "supernumerary chromonenal reproduction" or "endomitotic polyploidization" has also been observed by Geitler (9) and Huskins and Steinitz (13) by following in the energic nucleus the fate of chromosomes or parts of chromosomes which are heterochromatic.

Jahnl (15), Lauber (16), and others have used tissue wounding to induce mitosis in differentiated plant tissues. Polysomatic nuclei have been observed in stems, in leaves, in fleshy fruits, and in root tissues. Other workers,

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notably Levan (17), Huskins and Steinitz (14), and D'Amato (4), have studied nuclear divisions induced by various growth substances, such as indoleacetic acid (IAA), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). They also observed polyploid nuclei in differentiated tissues of the roots of various plants.

It has been argued that the action of stimuli, such as wounding or the application of hormones, might be to induce polyploidy rather than merely to reveal it through mitosis. Thus Levan (17), in studying the cytological phenomena connected with subapical tumefaction of onion roots after continuous treatment for four to six days with 1 to 10 p.p.m. of different growth substances, pointed out that in their early divisions the giant cortical cells are diploid but that "at a certain stage" they become polyploid, the degree of polyploidy increasing with distance from the root apex. He concluded from these results that IAA and other growth substances have two main effects: a primary effect causing an increase in cell size, and, as a secondary response to the increased volume, endomitosis. He concluded that cell enlargement is thus in itself the stimulus to the doubling of the chromosomes whereas the initiation of mitosis is a secondary effect. Dermen (6), observing different degrees of polyploidy at the internodes of beans treated with naphthaleneacetic acid, concluded that the polyploid condition is induced by the growth substance as a result of an effect on the premitotic nuclei. On the other hand, Huskins and Steinitz (14), Berger (1), Coleman (3), and Therman (20) have supplied data which they interpreted as showing that these substances when suitably applied induce mitosis in cells which were diploid or polyploid previous to treatment.

Although Levan and Lotfy (18) concluded that tissue differentiation is often normally connected with endomitotic processes, partly because they found some naturally occurring endomitotic nuclei, they also state: "it is not yet decided whether the phytohormone induces the chromosome doubling or only reveals the occurrence of it by starting mitoses".

Any significance that the results obtained by the use of growth substances to induce mitoses may have for concepts of chromosome constitution in differentiated tissues depends on the mode of origin of the polyploid nuclei. The main part of the present work has been concerned with trying to discover whether nuclear differences in differentiated tissues are merely revealed through the induction of mitosis with growth substances, in particular IAA, or whether they are produced by the treatment itself.

Materials and Methods

White "pickling" onion bulbs weighing less than 15 gm. were rooted in distilled water which, although not artificially aerated, was changed every 12 hr. The bulbs were kept in the dark and at a constant temperature of 20°–21° C. Treatment began after three days when each bulb had 20–40 roots, up to 25 mm. long, and new ones were still appearing. Despite differences in age, length, and rate of growth, the roots were remarkably

uniform in width. The treatment consisted in setting the bulbs on 40-cc. vials of IAA solution. After treatment, each bulb was rinsed in running distilled water for about 10 seconds before it was placed over fresh distilled water.

The following experiment was designed not only for determination of the range of treatments effective in mitotic induction and the optimum treatments for stimulation of mitosis in diploid and polyploid cells, but also to yield information concerning relationship between diploid and polyploid cells in regard to mitotic induction at different distances from the root apex. In contrast to Levan (17, 18) and others, short durations of treatment were used and early effects of the growth substances were investigated in order to eliminate the possibility of secondary effects.

The variables in treatment were: (*a*) concentration of IAA (3, 9, and 27 p.p.m.), (*b*) duration of treatment (1, 4, and 16 hr.), and (*c*) time between initiation of treatment and fixation of roots (1, 4, 10, 16, 22, 28, 34, 40, 52, and 64 hr.); each time interval was applied to each combination of IAA concentration and treatment duration.

For each of the nine combinations of IAA concentration and duration of treatment, four bulbs were used (36 in all). At each of the 10 fixation periods four roots, one from each bulb, were fixed for squash preparations. (An additional root was fixed separately for sectioning at each of several fixation times, i.e., 16, 28, 40 and 64 hr.). Thus a total of 90 combinations of the three treatment variables were investigated.

In order to reduce inconsistencies in results due to intra- and inter-bulb variability, a careful randomization scheme was adopted throughout the experiments. First, each bulb was assigned at random to one of the different types of treatments. Furthermore, each root on each bulb was randomly selected in regard to time of fixation. The only type of selection practised was the discarding of any root that was not at least 15 mm. long at the scheduled time of fixation, for the sake of greater uniformity in regard to growth rate.

To reduce subjective bias and to counteract effects of possible shift of standards during the observations, all the fixed roots were given code numbers, uncoding being done upon completion of the scoring of all slides.

The roots to be used for squash preparations were fixed for 6 to 12 hr. in alcohol–acetic (3 : 1). The roots for sectioning were fixed for 24 hr. in Craf solution. Both types of material were stored in 70% alcohol until used. Feulgen squash preparations of the alcohol–acetic material were made as follows. Roots were hydrolyzed in *N* HCl for 12 min., stained in leuco-basic fuchsin, and cut under a dissecting microscope into five segments, 3 mm. long, beginning with the root apex. Several segments were squashed separately under one large coverslip in 45% acetic acid, care being taken to avoid disturbance of the sequence of the sections and to cause minimal upset in the arrangement of tissues. The material fixed in Craf was stained *in toto* in leuco-basic fuchsin and embedded in paraffin through the usual tertiary butyl alcohol series.

The microscopical observations involved the examination of the four root segments of each root in squash preparations and classification of all division figures found according to (*a*) root level, (*b*) stage of mitosis, and (*c*) whether diploid or polyploid. Sections were used to determine the tissues in which polyploid cells occurred and the distance from the root apex at which they first appeared.

Observations

(*1*) *Nuclear Types*

On the basis of the number of chromosomes and the number of visible strands per chromosome, three types of prophase nuclei can be clearly distinguished in differentiated tissues of onion root. (It should be kept in mind that the visible strands described in the present material are of the nature of chromatids and not of finer structural units. The chromatids are of the same diameter in all the prophase types.) In the first type the diploid number of chromosomes is present and each chromosome is composed of two chromatids (Fig. 1). In the second type the diploid number of chromosomes is also present but each chromosome consists of four chromatids all united at the centromere region (Fig. 2). In the third type the tetraploid number of chromosomes is present and each of them is composed of two chromatids (Fig. 3). In the first, second, and third cases the two or four chromatids separate from one another at metaphase (Figs. 4, 5, 6), and at anaphase a diploid (first type, Fig. 7) or tetraploid (second and third case, Fig. 8) group of chromosomes moves to each pole.

In addition to these three nuclear types, a fourth may also exist, for one prophase with apparently more than four chromatids per chromosome was observed. The chromatids were also considerably longer than normal (Fig. 10). Corresponding nuclei in the energic stage are characterized by an increase in size of their constituents, most noticeably the nucleolus and the "heterochromatic bodies" which are represented by exceptionally large bodies of densely stained material (Fig. 9).

In this work we shall refer to as diploid a prophase nucleus having the diploid number of chromosomes with each chromosome composed of two chromatids, and as polyploid a prophase nucleus having the diploid number of chromosomes each consisting of four or more chromatids or a prophase nucleus having the tetraploid number of chromosomes each consisting of two chromatids. All the subsequent tabulations and calculations are based on such a distinction.

It is significant, in connection with the question of induced polyploidy versus induced mitosis in already polyploid cells, that polyploid prophase nuclei with fewer than four chromatids per chromosome were extremely rare.

(*2*) *Localization of Nuclear Types*

Mitoses in differentiated tissues were localized as follows in sectioned material: In the control the diploid nuclei were found in the pericycle and the phloem; two tetraploid nuclei in late prophase occurred in the endodermis. In the treated roots the cytological picture, at least so far as the chromosome

numbers are concerned, is consistent for all the treatments used. In the endodermis the nuclei were diploid or polyploid at the lower levels of the root and almost all polyploid at the upper levels. Pericycle nuclei were generally diploid at all the root levels; only very rarely was a polyploid mitosis observed in this tissue. Protoxylem and phloem nuclei at the lower root levels sometimes divide but division in the upper part of the root is extremely rare. Dividing cells in these tissues were all diploid. Mitoses occurred occasionally in the metaxylem elements of the lower part of the root and all were tetraploid. In the central core of the root, i.e., in the immature metaxylem elements, the huge nuclei never seemed to undergo mitosis even after treatment with IAA, with the possible exception of a prophase already described which could be interpreted as an endoprophase, each chromosome of which had at least four chromatids that were considerably longer than normal (Fig. 10). This is apparently similar to a case described by Duncan and Ross (7) in corn endosperm nuclei, where not only the number of strands per chromosome but also the dimensions of the strands were increased.

Few mitoses were observed in the cortex following IAA treatment and then only toward the end of the observation period (52 to 64 hr.). All the mitoses in this tissue up to 64 hr. after the beginning of treatment were normal in respect to spindle formation and chromosome movement; some were diploid and some were polyploid. Nothing can be said about polyploidy in the epidermis, since it never reacts positively to treatment.

(3) Induction of Mitosis in Differentiated Tissues of the Root

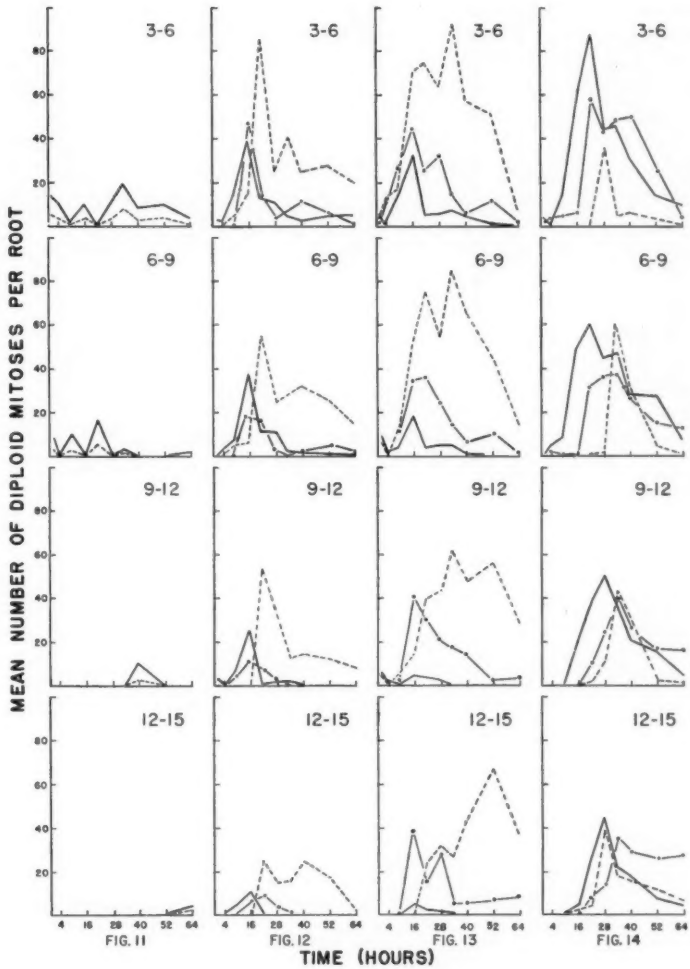
The nuclei at a given root level may be considered stimulated to divide as a result of the treatment when the frequency of mitoses becomes greater than the highest number of mitoses found in any of the controls at that level. The highest number of mitoses in the control was adopted as the criterion as a precaution against other than conservative conclusions. It should be stressed at this point that there is no significant change in the prophase/metaphase/anaphase divisions ratio at any root level or for any of the fixation periods for either diploid or polyploid divisions. The mitoses induced during the 64-hr. period after the beginning of treatment must therefore progress at a uniform speed throughout the fixation periods (up to 64 hr.) and IAA as applied in these experiments has no specific effects in shortening or lengthening individual phases of mitosis in either diploid or polyploid cells.

The following aspects of the induction of mitosis may be considered: (i) the lag period which became evident between the initiation of treatment and the beginning of mitotic induction, and (ii) the duration of mitotic induction.

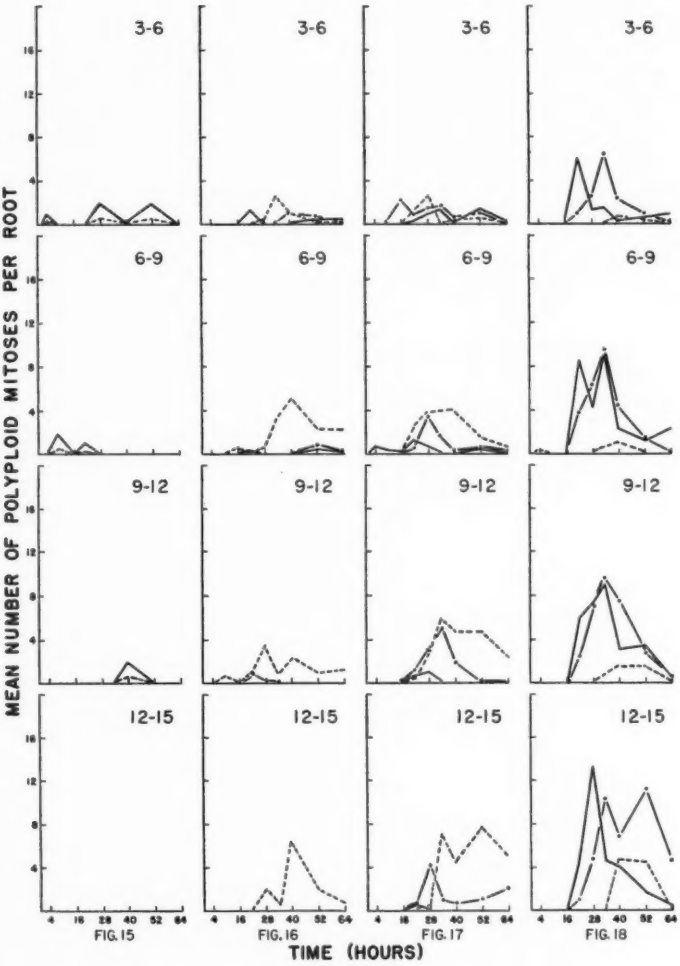
(i) Lag Period Before Mitotic Induction

The term "lag period" is applied here to the length of time that elapsed between the beginning of treatment and the time at which mitoses appeared in greater numbers than were found in the controls. In Figs. 11, 12, 13, and 14 the mean numbers of diploid and in Figs. 15, 16, 17, and 18 the mean numbers

FIGS. 1–10. × 1250. FIG. 1. Mid prophase with diploid number of two-chromatid chromosomes. FIG. 2. Mid prophase with diploid number of four-chromatid chromosomes. FIG. 3. Late prophase with tetraploid number of two-chromatid chromosomes. FIG. 4. Metaphase with diploid number of two-chromatid chromosomes. FIG. 5. Metaphase with diploid number of four-chromatid chromosomes. FIG. 6. Metaphase with tetraploid number of two-chromatid chromosomes. FIG. 7. Anaphase with diploid number of two-chromatid chromosomes. FIG. 8. Anaphase with tetraploid number of two-chromatid chromosomes. FIG. 9. Giant nucleus in the energic stage. Note prominent nucleolus and the large heterochromatic bodies. FIG. 10. Corresponding nucleus (Fig. 9.) in prophase with more than four chromatids per chromosome. The chromatids are also considerably longer than normal.



FIGS. 11, 12, 13, and 14. Variation in frequency of diploid mitoses at different root levels (3–6, 6–9, 9–12, 12–15 mm. from the root apex) with (1) different concentrations of indoleacetic acid (FIG. 11, control; FIG. 12, 3 p.p.m.; FIG. 13, 9 p.p.m.; FIG. 14, 27 p.p.m.), (2) different durations of treatment (———— 1 hr.; · ——— · 4 hr.; – – – – 16 hr.), and (3) different times of fixation after beginning of treatment (1, 4, 10, 16, 22, 28, 34, 40, 52, and 64 hr.). The solid line in control (Fig. 11) indicates the highest absolute number of diploid mitoses ever observed per root.



FIGS. 15, 16, 17, and 18. Variation in frequency of polyploid mitoses at different root levels (3–6, 6–9, 9–12, 12–15 mm. from the root apex) with (1) different concentrations of indoleacetic acid (FIG. 15, control; FIG. 16, 3 p.p.m.; FIG. 17, 9 p.p.m.; Fig. 18, 27 p.p.m.), (2) different durations of treatment (———— 1 hr.; · ——— · 4 hr.; – – – – 16 hr.), and (3) different times of fixation after beginning of treatment (1, 4, 10, 16, 22, 28, 34, 40, 52, and 64 hr.). The solid line in control indicates the highest absolute number of polyploid mitoses ever observed per root.

of polyploid divisions per root are plotted against time of fixation, in separate curves for each of the different treatments and for each of the four root levels studied (3–6, 6–9, 9–12, 12–15 mm. from the root apex). The lag periods for each combination of treatment and root level can be seen by comparison of the control curves (Figs. 11 and 15) with those of treated roots in Figs. 12, 13, and 14 for diploid divisions and in Figs. 16, 17, and 18 for polyploid divisions. These lag periods are presented in Table I for diploid and polyploid nuclei.

It appears that, in diploid nuclei, there is in general a relationship between the duration of treatment and the duration of the lag period between the beginning of treatment and the onset of mitotic induction. No explanation can be suggested for the possible inconsistencies, particularly in regard to the lag periods at the lower root level following treatment with 3 and 9 p.p.m. IAA for 16 hr. That the concentration of IAA is also a factor in the lag period is noted when the effects of 3 and 9 p.p.m. are compared with those of 27 p.p.m. It is also evident that with those dosages a minimum of 10–16 hr. after the beginning of treatment was required for any expression of mitotic stimulation.

TABLE I

LAG PERIODS IN HOURS BETWEEN BEGINNING OF TREATMENT AND ONSET OF MITOTIC INDUCTION

Concentration of IAA in p.p.m.	Duration of treatment in hr.	Distance from root apex in mm.			
		3–6	6–9	9–12	12–15
Diploid nuclei					
3	1	10–16	10–16	*	*
	4	10–16	10–16	*	*
	16	16–22	16–22	16–22	16–22
9	1	10–16	10–16	*	*
	4	10–16	10–16	10–16	10–16
	16	10–16	10–16	16–22	16–22
27	1	10–16	10–16	10–16	10–16
	4	16–22	16–22	16–22	16–22
	16	22–28	28–34	28–34	22–28
Polyploid nuclei					
3	1	†	†	†	†
	4	†	†	†	†
	16	†	28–34	22–28	22–28
9	1	†	†	†	†
	4	†	22–28	22–28	22–28
	16	†	22–28	22–28	28–34
27	1	16–22	16–22	16–22	16–22
	4	28–34	22–28	22–28	22–28
	16	†	†	†	34–40

* *The number of diploid mitotic figures was not greater than in the controls.*
† *The number of polyploid divisions was not greater than in the controls.*

As in the case of diploid divisions, the lag period for polyploid divisions was extended in most instances when the duration of treatment was prolonged. The effect of the concentration on the lag period cannot be studied as readily in the polyploid divisions as in the diploid because of failure of any mitotic induction with less severe treatments. However, at the 12–15 mm. level a consistent increase in lag period with increased IAA concentration is noted. The inconsistencies can probably be ascribed primarily to the fact that the numbers of polyploid mitoses were relatively small.

Comparison of lag period for diploid and polyploid divisions shows that for all the treatments except 27 p.p.m. for 4 hr. at the upper root levels there is a 6- to 12-hr. period between the induction of diploid divisions and that of polyploid mitoses. The significance of this will be considered later. Furthermore, it is apparent that certain treatments induce diploid divisions without inducing polyploid divisions. This will also be discussed later.

(*ii*) *Duration of Mitotic Induction*

Duration of mitotic induction is the length of time during which the frequency of mitosis remains greater than the highest found in any control root. The durations of mitotic induction for each combination of treatment and root level can be seen by comparison of the control curves (Figs. 11 and 15) with those of treated roots in Figs. 12, 13, and 14 for diploid and in Figs. 16, 17, and 18 for polyploid. These durations of mitotic induction are presented in Table II for diploid and polyploid nuclei.

With the concentration of IAA that proved most effective in inducing mitosis in diploid cells (9 p.p.m., as will be shown later), there is a direct relationship between duration of treatment and duration of mitotic induction. The same trend is noted when the concentration was 3 p.p.m., although the difference between one and four hours was apprently not sufficiently long to affect induction duration. With 27 p.p.m. an inverse relationship existed between duration of treatment and length of the induction period, which may be connected with increasing toxicity of that IAA concentration with prolongation of treatment. Similarly, the shorter duration of mitotic induction at the lower root levels is considered to reflect the more toxic effect of 27 p.p.m. there than at the upper levels.

In the case of polyploid divisions, the correlations between durations of treatment and of mitotic induction are not so clear-cut, probably because of the relatively small numbers of polyploid divisions dealt with. Nevertheless, there is some indication of increased duration of mitotic induction with increased length of treatment. Also, a tendency is noted toward increased duration of mitotic induction with increasing distance from the root apex.

(*4*) *Relationship Between Diploid and Polyploid Cells in Their Response to Indoleacetic Acid*

(*i*) *Relative Effectiveness of the Different Treatments*

One way of evaluating relative effectiveness of the different treatments is through comparison of total numbers of diploid and polyploid mitotic figures

induced by a specific treatment at a given level of the root during the whole period of observation (from 1 to 64 hr. after the beginning of the treatment), regardless of time of onset and duration of mitotic induction. Estimates of that effectiveness were obtained in the following manner: First, the mean number of either diploid or polyploid mitoses per root level and at each fixation period was calculated. Second, for each level of the root these averages for all the fixation periods and after treatment up to 64 hr. were added. Since we are interested primarily in a comparative analysis of the effectiveness of the different treatments no correction for the relatively small number of mitoses observed in the control appeared necessary. The sums of the means are plotted against distance from the root apex in Fig. 19 in separate curves for three treatments that proved to be most effective in inducing mitoses.

Fig. 19 shows that the treatment 9 p.p.m. for 16 hr. is the most effective (of those used) for inducing diploid divisions, with 27 p.p.m. for 1 hr. the next most successful treatment; 27 p.p.m. for 4 hr. is, however, the most effective treatment for inducing divisions in polyploid cells, and 27 p.p.m. for

TABLE II

DURATION OF MITOTIC INDUCTION IN HOURS

Concentration of IAA in p.p.m.	Duration of treatment in hr.	Distance from root apex in mm.			
		3–6	6–9	9–12	12–15
Diploid nuclei					
3	1	10	10	*	*
	4	10	10	*	*
	16	45	45	45	45
9	1	10	10	*	*
	4	22	22	28	22
	16	50	50	50	50
27	1	40	45	45	45
	4	35	35	45	45
	16	10	16	22	35
Polyploid nuclei					
3	1	†	†	†	†
	4	†	†	†	†
	16	†	34	16	34
9	1	†	†	†	†
	4	†	10	16	40
	16	†	28	34	34
27	1	10	45	40	45
	4	16	34	34	45
	16	†	†	†	34

* *The number of diploid divisions was not greater than in the controls.*
† *The number of polyploid divisions was not greater than in the controls.*

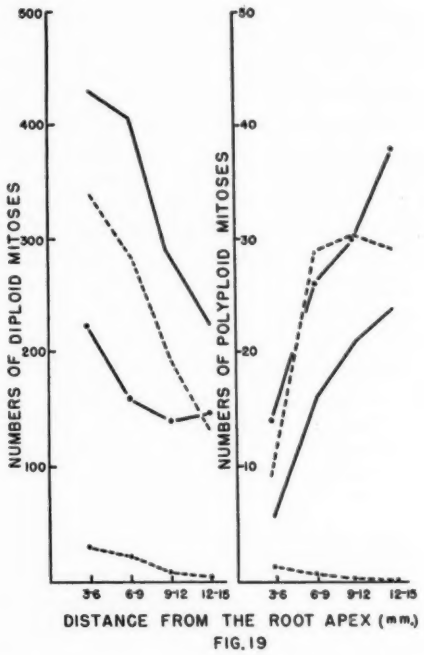


FIG. 19

FIG. 19. Total effectiveness of three treatments (· ——— · control; ——— 9 p.p.m. indoleacetic acid for 16 hr.; - - - - 27 p.p.m. for 1 hr.; · ——— · 27 p.p.m. for 4 hr.) in stimulating mitosis in diploid and polyploid cells at various distances from the root apex (3–6, 6–9, 9–12, and 12–15 mm.).

1 hr. and 9 p.p.m. for 16 hr. are the next most effective treatments in that sequence. The other treatments (not indicated in Fig. 19) 3 p.p.m. for 1 and 4 hr. as well as 9 p.p.m. for 1 and 4 hr. have practically no effect on polyploid nuclei but cause significant increase in numbers of diploid divisions, at least at the lower root levels. The treatment 27 p.p.m. for 16 hr. has toxic effects on the root tissues, particularly at lower root levels, which applies to both diploid and polyploid cells, although it causes relatively less effect on the latter than on the former at higher root levels. Similarly, the toxicity of 27 p.p.m. for 4 hr. is greater for diploid cells at the three lower root levels than at the upper level. From these facts the conclusion is made that polyploid nuclei not only tolerate higher doses of IAA than the diploid, but actually require higher doses for induction of significant numbers of divisions.

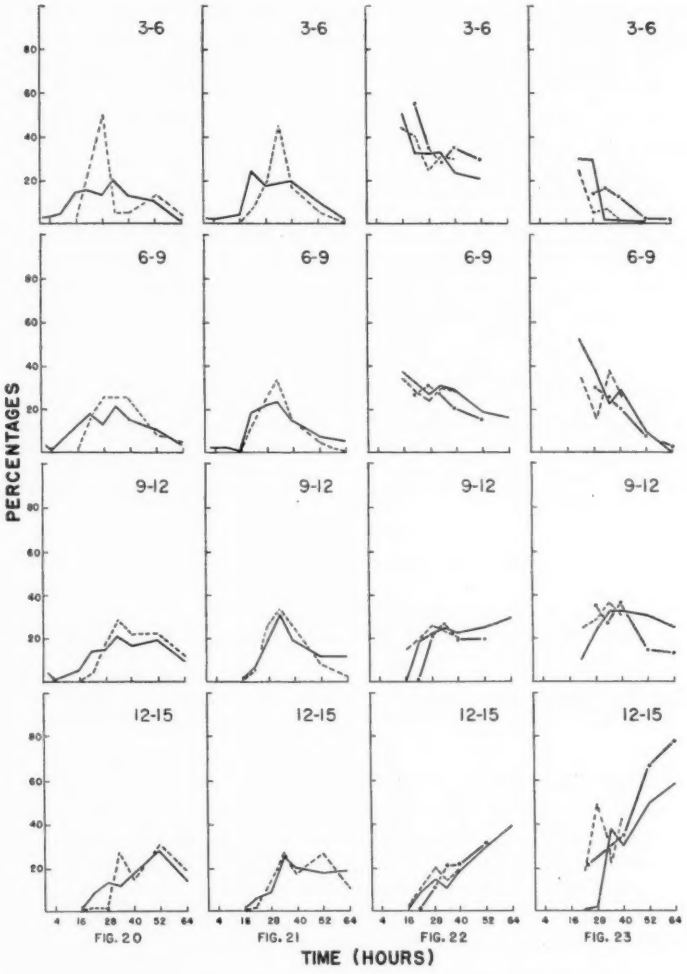
Fig. 19 also shows that whereas all effective treatments have progressively less effect on successively higher root levels in regard to mitotic stimulation in diploid cells, the reverse situation is seen for polyploid cells, where all effective treatments produce increasingly greater effects as distance from the root apex increases. The conclusions drawn from this are the following:

(1) the number of polyploid cells increases with distance from the root apex; (2) though there may be fewer diploid cells at the upper levels, because some cells have become polyploid, this is probably not enough to account for the great difference in diploid mitotic induction at the lowest and highest levels following the most generally successful treatments. These points will be discussed later.

(ii) Relative Proportions of Over-all Effectiveness of Treatment

If for a given root level the over-all effectiveness of a given treatment (which includes IAA concentration and duration of treatment) in stimulating mitosis is represented by the sum of the mean numbers of diploid (or polyploid) nuclear divisions for all fixation periods, i.e., from 1 to 64 hr. after the beginning of treatment, then the percentage of diploid (or polyploid) mitoses achieved at each time of fixation represents the per cent effectiveness of that treatment at that fixation time and at that level. By using the data in this way it is possible to determine whether effectiveness of treatment, as defined above, progresses in time in the same manner for both diploid and polyploid divisions—in other words, whether at a given hour of fixation and at a given root level the same fraction of over-all effectiveness has been reached for the two classes of nuclei. Comparisons of diploid versus polyploid cells in regard to the progression of effectiveness with time at each root level can be made in Figs. 20 and 21 for the two treatments which induced the greatest number of both diploid and polyploid divisions (9 p.p.m. for 16 hr. and 27 p.p.m. for 4 hr.). With 9 p.p.m. for 16 hr. (Fig. 20) a higher proportion of effectiveness is achieved in the diploid than in the polyploid nuclei at all the root levels at the beginning of mitotic induction, i.e., when the curves start rising steeply. The relationship applies also to the effects of the treatment 27 p.p.m. for 1 hr. (not indicated here) except at the highest level, where onset of induction occurs at approximately the same time in both diploid and polyploid cells and progression of induction is therefore almost identical for both. With the most severe treatment, 27 p.p.m. for 4 hr. (Fig. 21), the close approximation of the two curves, particularly at onset of induction, is seen at all but the lowest level. In all the treatments studied, at the 3–6 mm. root level a relatively high proportion of diploid divisions is achieved earlier than in the case of the polyploid and, with some fluctuation, tends to be maintained for most of the induction period. By contrast, the proportions of polyploid divisions at the 3–6 mm. root level from the root apex increase rapidly in a much steeper rise, then decrease rapidly. At the upper root levels in general, proportions of divisions in polyploid cells more closely parallel those of diploid cells, although the peaks in the former are generally higher and the onset of induction later. This is particularly striking in view of the fact that the calculations were based on comparatively small numbers of polyploid divisions.

Although, as shown previously, response of diploid and of polyploid nuclei to various aspects of treatments are different at some point in the range of treatments used, mitotic induction (at all but the 3–6 mm. level) begins at



FIGS. 20 and 21. Proportions of over-all effectiveness achieved for diploid (————) and for polyploid (- - - -) mitoses in various root levels (3–6, 6–9, 9–12, and 12–15 mm. from the root apex) and at different times after beginning of treatment. FIG. 20 is for treatment 9 p.p.m. indoleacetic acid for 16 hr. and FIG. 21 for treatment 27 p.p.m. for 4 hr. In ordinates are the percentages of the mean number of diploid (or polyploid) divisions at each fixation time, based on the total mean numbers of diploid (or polyploid) mitotic figures at each level during all the fixation periods.

FIGS. 22 and 23. Progression of mitotic induction from lower to higher root levels with various treatments (———— 9 p.p.m. indoleacetic acid for 16 hr.; - - - - 27 p.p.m. for 1 hr.; · ——— · 27 p.p.m. for 4 hr.). In ordinates are the percentages of the sum of means of diploid (FIG. 22) or polyploid (FIG. 23) mitoses at all root levels represented by the mean number at each root level. In abscissa is the time of fixation in hours after the beginning of treatment.

the same time after initiation of treatment in both diploid and polyploid nuclei and the proportions of effectiveness are apparently the same for both throughout the rest of the observation period. This is taken to indicate that the differences in chromosome number were pre-existing.

(*iii*) *Progression of Mitotic Induction*

To trace the progression of mitotic induction with time the percentage of the mean number of diploid (or polyploid) mitotic figures at a given level and at a given time of fixation was calculated from the sum of the mean numbers of diploid (or polyploid) divisions at all the root levels at that fixation time. Such calculations were made for only those treatments which produced relatively large numbers of both diploid and polyploid divisions at all the root levels (9 p.p.m. for 16 hr., 27 p.p.m. for 1 hr., and 27 p.p.m. for 4 hr.) but only at fixation times at which the frequency of mitoses was greater than the control. It is for this reason that the curves are not all of the same length.

The percentages were plotted against time of fixation in Fig. 22 (diploid mitoses) and Fig. 23 (polyploid mitoses). With 9 p.p.m. for 16 hr. at the beginning of mitotic induction the proportions of diploid divisions at the root levels closer to the root apex were greater than those at the upper levels, whereas at the end of the induction period the proportion was considerably greater in the upper levels of the root. The same applied to the other two treatments, i.e., at the beginning of induction the proportion of divisions was relatively high in the lower levels and low in the upper levels but at the end of the induction period the situation was reversed. In spite of the relatively small numbers of polyploid divisions from which the calculations were made, the same relationships existed as that described for the diploid mitotic figures (Fig. 22). The similarity between the progression of mitotic induction in diploid cells and that in polyploid cells at different root levels and its possible significance will be considered in the next section.

Discussion

As stated previously, the primary objective of this investigation was to establish more definitely than has previously been done whether differences in chromosome number exist normally in differentiated tissues of *Allium cepa* roots and are merely revealed by treatment with growth substances which induce mitosis, or whether those nuclear differences are produced by the treatment. Evidence that chromosome number differences exist in the differentiated tissues of onion prior to any treatment and that their appearance after treatment is a matter of revelation and not of causation has been obtained in these investigations and is presented in what follows.

(i) Differences in chromosome number in nuclei of normal differentiated tissues can be assessed indirectly in some cases from observation of nuclear size and structure or directly from observation of rare spontaneous mitoses. In the former category are the giant nuclei in the central part of the root (Fig. 9, metaxylem) which on the basis of size and structure were judged to

be polyploid. Also, in the cortex nuclear sizes indicate the probability that some cells, particularly at upper root levels, are polyploid. Correlating these observations in controls with comparable ones in treated roots, we find that the single giant cell induced to divide (Fig. 10) was definitely polyploid and furthermore had apparently achieved a higher degree of polyploidy than the tetraploid state, since the number of strands per prophase chromosome was more than four. (As mentioned before, a nucleus is considered polyploid either if it contains a multiple of the diploid number of chromosomes each having four or more visible strands or if it includes the tetraploid number of two-chromatid chromosomes.) Duncan and Ross (7) found that with an increase in the size of a maize endosperm nuclei, although there is no increase in the number of chromosomes, an increase does occur in the number and/or in the dimensions of discernible strands making up a chromosome. It is believed that a similar situation probably exists in the nuclei of the metaxylem elements of onion roots. Perhaps these giant nuclei represent polyploidy in a different aspect from that in polyploid cells of other tissues since the former are characterized not only by greater numbers of chromatids per chromosome but also by more rapid chromatid reproduction. Furthermore, it is extremely difficult to induce them to divide. Similarly, Duncan's (7) attempts to induce division in maize endosperm nuclei through IAA treatment were completely unsuccessful. Finally, their structure in the energic stage is different from that of nuclei in other tissues: their nucleoli and heterochromatic bodies reach enormous proportions. These are considered probable indications of exceptional increase in number of chromosome strands.

Direct evidence of polyploidy in differentiated tissues was provided by spontaneous mitoses in control roots, for 12 cells undoubtedly contained the tetraploid chromosome number. Six were prophases with four-chromatid chromosomes, four were tetraploid metaphases, and two were tetraploid anaphases. Levan and Lotfy (18) also found spontaneous mitoses with diplochromosomes (four-chromatid chromosomes) in tissues of onion roots. That polyploid cells are not limited to the root in *Allium* is shown by the fact that spontaneous mitoses in polyploid cells of the cotyledon mesophyll and in the cortex of the transition region were found by Berger and Witkus (2) in untreated plants.

(ii) It is apparent that treatment with any of the effective IAA dosages merely increases the numbers of dividing nuclei, polyploid as well as diploid, as compared with numbers in the controls. The chromosome structure was the same in treated roots as in the controls. Found in both treated and control roots were two types of prophase nuclei—those with the diploid number of two-chromatid chromosomes and those with the diploid number of four-chromatid chromosomes. Rarely in treated material prophases with the tetraploid number of two-chromatid chromosomes were found. Such propases undoubtedly occurred in cells which had undergone a previous division after attaining the tetraploid condition.

In the treated material there is no indication that the polyploid nuclei might have arisen by "restitution" in abortive mitoses either before, during,

or after treatment nor of any colchicine-like effect from the treatment. The fact that there is no significant change in the prophase/metaphase/anaphase (P/M/A) ratio during the mitotic induction shows that induced mitosis in polyploid cells proceeds in a normal manner. Since polyploidizing agents such as colchicine disrupt the normal P/M/A ratio and cause chromosome doubling through abnormalities, such as failure of spindle formation, the normal P/M/A/ ratio following IAA treatment is considered evidence that IAA (with the durations of treatment used here) induces mitosis, not polyploidy. The nuclear abnormalities reported as normally occurring in the "old" cortical tissues of *Allium cepa* root tissues by D'Amato and Avanzi (5), Levan and Lotfy (18), and D'Amato (4) and as resulting from "chromosomal decay in aging tissues" are believed to be the result of their prolonged treatment with naphthalene acetic acid (10-day treatment) or 2,4-D (three-day exposure). If mitoses in these tissues are observed two or three days after the beginning of treatment with IAA or NAA both diploid and polyploid mitoses are normal.

Since the completion of the present work, evidence has been presented by Naylor (19) strongly suggesting that in tradescantia axillary buds released from dormancy, auxin initiates DNA synthesis in the energic nuclei before inducing them to divide. If DNA synthesis materializes in an increase in the number of strands per chromosome (as is the case in ordinary root meristem), it becomes difficult to separate the increase from one to two chromatids per chromosome which follows release from inhibition and the increase from two to four chromatids per chromosome at the time of endomitosis. The difference, of course, is that mitosis in one case reduces the number of strands per chromosome and does not in the other. Perhaps both the nuclei capable of division in newly active buds and the nuclei which had hitherto been endomitotic double their amount of DNA (yet to be checked in the latter case) and double the number of strands per chromosome. When they divide, of course, the number of strands per chromosome is reduced to the condition before stimulation.

No matter what decision is reached about IAA and the initiation of DNA synthesis, Naylor (19) did not show that the application of IAA causes polyploidy and his result bears out our work in this regard.

(iii) In spite of the fact that the range of treatment was so extensive that at one end of the range practically no effect resulted and at the other the toxicity of IAA inhibited divisions, the chromosome numbers of the different tissues form a consistent pattern irrespective of severity of IAA treatment. Thus, the meristem remained diploid even in a few roots that in spite of relatively high dosages continued to be mitotically active to the end of the observation period (64 hr.). In the differentiated tissues also, the evidence is opposed to the assumption on the part of some workers that IAA induces polyploidy. The pericycle remained diploid at all the root levels. By contrast, in the endodermis polyploid cells began to appear at approximately 6–9 mm. above the root apex and consistently increased in numbers with

increasing distance from that root level. If IAA did induce polyploidy, it would be difficult to explain why the same treatment that induced only diploid mitoses in the pericycle could at the same time cause polyploidization in the adjacent endodermis as well as why the treatment polyploidized the endodermis only in the upper root levels and not in the lower ones. Whether the cells in both or either of these two tissues are induced to divide after treatment with IAA depends on the concentration of the growth substance, duration of treatment, and distance at which the observations are made from the root apex. The few mitoses induced in the phloem and protoxylem at the lower root levels were diploid, while those in the metaxylem elements, aside from the giant nuclei in the central part of the root, were tetraploid. At upper levels the cells in these phloem and xylem areas are rarely induced to divide. It is believed that the effects of the different treatments with IAA varied only in the numbers of divisions induced in each tissue and at different root levels.

(iv) Considering the treatments which are most effective in inducing mitosis at all the root levels, if diploid and polyploid cells are normally present in differentiated tissues and if IAA merely induces mitosis, it should be expected that whenever mitotic induction is great, it should be proportionally intense in diploid and polyploid cells and when mitotic induction decreases it should decrease proportionally in diploid and polyploid cells. Comparison of percentages of total effectiveness in induction at different levels and fixation periods shows that this expectation in general is fulfilled, since approximately the same percentages of total effectiveness for both diploid and polyploid mitoses occur throughout the induction period at the upper three root levels, except at the beginning of mitotic induction. The existence of a definite lag period between the beginning of induction in diploid and in polyploid cells and its complete disappearance with certain treatments is according to expectations for the following reason: in studying the relative effectiveness of the different treatments in stimulating mitosis in diploid and polyploid cells, it was shown that polyploid cells not only tolerate higher doses of IAA than diploid cells, but actually require higher doses for induction of significant numbers of divisions. Therefore, the level of IAA necessary to stimulate mitosis in diploid cells is reached earlier in the treatment than that required to induce polyploid divisions. It is also possible that a slower division rate in polyploid than in diploid cells also contributes to the lag period. Absence of a lag period between induction of diploid and polyploid mitoses following the more severe treatments is attributed to delayed division of diploid cells resulting from slight toxic effects of those treatments, which reduce the numbers of diploid mitoses as well.

At the 3–6 mm. level, however, the relatively close relationship found between the percentage curves for diploid and polyploid mitoses at the higher root levels was not found with any treatment used. One explanation may be that at that level the number of polyploid cells is relatively small compared with those at upper levels and perhaps the majority are induced to divide at

approximately the same time. This would explain the steep rise in percentages for polyploid divisions and its almost immediate drop thereafter.

Additional evidence from proportions of diploid and polyploid divisions pointing to IAA induction of mitosis but not polyploidy is found in the parallel progression of induction in diploid and in polyploid cells. At the beginning of mitotic induction, the highest proportions of both diploid and polyploid divisions occurred at the root levels closest to the root apex, while towards the end of the observation period, the largest proportions of mitoses (both diploid and polyploid) are found at the upper root levels. The similarity in progression in diploid and polyploid cells is particularly striking in view of the fact that the numbers of polyploid mitoses were, in comparison with the diploid, extremely small with all the treatments studied.

(v) If the growth substance acted as a polyploidizing agent, it might be expected that the greatest number of polyploid divisions would be found at those root levels where mitotic activity was the greatest during the 64-hr. period of observation, assuming that mitotic activity during that period is an indication of treatment effectiveness in all respects. With treatments which induced relatively large numbers of both diploid and polyploid mitoses, the greatest numbers of mitoses were found at the 3–6 mm. level, the numbers decreasing with increasing distance from the root apex. However, the numbers of polyploid mitoses were with all treatments smallest at the 3–6 mm. root level and increased with increasing distance from the root apex. In other words, the numbers of polyploid divisions were always higher at the upper root levels—regions of least mitotic activity—than at the lower ones as soon as and as long as mitoses were induced at all levels. From this, it is concluded that the number of polyploid cells increases with distance from the root apex. The decrease in number of diploid mitoses with distance from the root apex is only partially accounted for by chromosome doubling in cells that were diploid when closer to the root apex. Some loss to the ranks of diploid mitoses probably resulted from degeneration of nuclei accompanying differentiation in certain type of cells, e.g., some xylem and phloem elements. It is possible that some diploid nuclei in the regions of greatest mitotic activity might pass through more than one mitotic cycle during the 64-hr. observation period, which may also be a factor in the greater number of diploid mitoses at the lower root levels. The possibility of two mitotic cycles in polyploid cells during the 64-hr. observation period is, however, unlikely since even at the end of the observation period tetraploid prophases with two chromatids per chromosome were extremely rare.

For all these reasons, we consider that in certain tissues there is a gradient of spontaneous endomitotic polyploidization along the longitudinal axis of the onion root, which is merely revealed by treatment with indoleacetic acid.

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ECOLOGICAL STUDY OF THE PEAT BOGS OF EASTERN NORTH AMERICA

II. THE *CHAMAEDAPHNE CALYCULATA* COMMUNITY IN QUEBEC AND ONTARIO[1]

By Fernando Segadas-Vianna[2]

Abstract

Data collected in this very uniform bog community lend themselves particularly well to an assessment of phytosociological criteria. Expressions of frequency, constancy, fidelity, homogeneity as proposed by various writers are tested and evaluated. At the same time, it is believed that the present paper provides an accurate description and definition of the phytosociological unit with which it is concerned.

Introduction

The present study was made possible thanks to a grant from the National Research Council of Canada during the years 1947 and 1948, for a general study of the bogs in eastern Canada, under the direction of Dr. Pierre Dansereau.

The larger part of Quebec Province, with the exception of the territory east of Lake St. John and the Arctic, and the northeast of Ontario Province were studied during our researches, which were limited to the vegetation and its dynamism.

I am most grateful to Dr. Pierre Dansereau, under whose direction this research was done, for his encouragement and for the facilities made available to me at the Service de Biogéographie (Université de Montréal), and to Dr. Stanley A. Cain for his helpful suggestions. I also wish to thank Mr. Edgar Kuhlman for his co-operation in field work and without whose help it would have been difficult to execute the project; to Mr. A. Courtemanche and Dr. Lucien Lévesque, c.s.c., for their aid and suggestions; to Dr. W. C. Steere, who identified the mosses, and, finally, to my wife Carmen Dulce Segadas-Vianna, for her help in translating the manuscript.

I wish also to express my appreciation for the time and facilities provided by the Cranbrook Institute of Science during the preparation of this manuscript. I am also indebted to Dr. Christovam Leite de Castro, then secretary-general of the Conselho Nacional de Geografia (Rio de Janeiro, Brazil) who gave me leave to study in Canada and the United States from 1947 to 1949, while I was a research assistant of that institution.

General Considerations

The *Chamaedaphne calyculata* association is, no doubt, one of the most widespread and characteristic of the American bog associations. It generally

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occupies large areas (Fig. 1), and because of the wide tolerance spectrum of *Chamaedaphne calyculata*, it is quite long-lived and could be in some cases considered as comparatively stable, its persistence being due to inhibiting edaphic conditions. As a matter of fact, this species is probably more than any other a characteristic bog species, for besides being found in almost all of the boreal bogs and being exclusive to the bog habitats, it can tolerate the most diverse influences, such as drainage and fire. Its high vegetative reproductive capacity allows a rapid and extensive establishment of almost pure stands in which the settlement of invading species becomes difficult.

Many surveys were made during the investigation, but only 11 were used in the final draft, the others having not been utilized for the represented progressive or regressive degradation phases, or association mosaics. The number of stations that contained characteristic examples of the association, and the small extent of the research area, did not allow the suitable delimitation and definition of the various geographical, edaphic, and degradation facies that probably exist. Another difficulty is that the number of constituent species of the association is so small that any important shift in the value of the species cover changes it into another association. On the related associations of *Kalmia angustifolia*, *Ledum groenlandicum*, and *Eriophorum spissum* (Dansereau and Segadas-Vianna (16)), we do not have enough data to allow an exact delimitation at the present time.

The author, having decided to test the value of some phytosociological concepts, chose four bogs in the province of Quebec, situated in Farnham, Lanoraie, Bellerive, and Nominingue, to be studied in detail.

Lanoraie bog, 40 miles northeast of Montreal, in the lowlands of the St. Lawrence Valley, is crossed by tracks of the Canadian Pacific Railway. Its area is about 7500 acres, of which some 3000 acres are occupied by the *Chamaedaphne* association. The rest of the area is covered by various associations, among which the most important is that of *Populus tremuloides*. A large part of the bog was burned several times in the last 10 years, including the part occupied by the *Chamaedaphne* association. The bog surface is quite irregular on the site of the *Chamaedaphne* community, the drainage being very poor. Great quantities of sphagna are taken annually by the nursery of the provincial government which is situated a short distance away. This intervention transforms the floristic composition of the inferior stratum.

Farnham bog is situated 1 mile East of the Ste. Brigide station and 3 miles West of Farnham in the Richelieu lowlands. Its area reaches 5100 acres of which about 1500 are occupied by *Chamaedaphne*. The larger part of this area was burned often during the last 10 years. This, together with industrial extraction of peat in several parts, changes the structure and composition of the vegetation considerably. The sphagna attain a large development in the *Chamaedaphne* association, in some places submerging individuals of *Chamaedaphne*, *Ledum groenlandicum*, and *Kalmia angustifolia*. Because of this the surface is very irregular and so is the drainage, as the *Sphagnum* cushions reach an average height of 60 cm.

Bellerive and Nominingue bogs, located in Labelle County on the laurentian shield, have areas approximately of 400 and 2000 acres. In both, fire in its recent passage has modified the general vegetation considerably, but has not changed the *Chamaedaphne* community. The bog surface here is rather regular and the drainage is relatively poor. The sphagna do not reach an optimum development as in the two other bogs.

Structure of the Association

In a well-developed stand of *Chamaedaphne*, one may distinguish two strata and, sometimes, even three. A preliminary description has already been given (Dansereau and Segadas-Vianna (16)), which will be enlarged upon here. The superior stratum is formed almost exclusively of *Chamaedaphne calyculata*. Its height is generally 40 to 60 cm., rarely reaching 1.30 meters; its total cover varies between 40% and 100% (see Table III, surveys 5 and 11), high coverages occurring most of the time.

Chamaedaphne calyculata is a semideciduous shrub that grows in groups of several individuals, with superficial fibrous roots and without any nodules, which is very uncommon among bog plants (Gates (22)). Its root system, according to Gates, seems to be insufficient during winter (p. 239), and the ends of its shoots generally die on account of the frosts (Gates (21, 22)).

With *Chamaedaphne* grow several shrubs, such as *Ledum groenlandicum*, *Kalmia angustifolia*, *Kalmia polifolia*, *Andromeda glaucophylla*, and *Rhodora canadensis*. In some stations, one or more of those species become co-dominants, as in surveys 1, 7, and 11 (see Table III).

The second layer, when present, is constituted of *Vaccinium angustifolium* or of one of the *Carex* species (*C. disperma*, *C. trisperma*), or occasionally of *Eriophorum spissum*. The latter occurs mainly after the passage of fire. This synusia is rarely distinct, because of the competition for light.

Different species of *Sphagnum* cover the ground and in some stands, as Farnham and Lanoraie, develop so that they finally submerge the individuals of *Chamaedaphne*, from which only the ends of the branches show. The *Chamaedaphne* thus covered forms large cushions that reach, in some places, from 60 cm. to 1 meter in height. In the depressions between the cushions occur several plants, the most common being *Vaccinium oxycoccos*, *Smilacina trifolia*, *Drosera rotundifolia*, and *Sarracenia purpurea*. The cover of the *Sphagnum* layer is generally 100%, but in certain stations it may reach only 10% and even be completely absent (see Table III, surveys 2 and 7). This situation is common in southern Michigan but rather rare in eastern Canada. The absence of the *Sphagnum* layer seems to indicate old stands of the *Chamaedaphne* community.

In addition to *Sphagnum*, one finds several mosses and lichens, the most common being *Polytrichum strictum*, *Polytrichum commune*, *Aulacomnium palustre*, *Pleurozium schreberi*, *Cladonia rangiferina*, *Cladonia pyxidata*, and *Cladonia pyxidata* var. *chlorophaea*. In some stations *Polytrichum strictum*

becomes co-dominant (Table III, survey 4) and sometimes dominant (Table III, survey 8), replacing *Sphagnum*. What is interesting to observe is the fact that when *Polytrichum strictum* is dominant its total cover is not low, as for example survey No. 8 where, in an area of 25 square meters, it presented a total cover of 80%. *Pleurozium schreberi* and *Aulacomnium palustre* have generally a very low cover, never more than 10% of the total area.

In some stands, *Sphagnum* is completely covered with a layer of *Vaccinium oxycoccos*, which is in general limited to the depressions among the cushions. In those places are found individuals of *Vaccinium oxycoccos* that may reach 40 cm. of length, tangling themselves on the low branches of *Chamaedaphne*.

One of the most conspicuous bog plants is *Sarracenia purpurea*, usually rare in the *Chamaedaphne* community and, when present, found generally grouped in colonies situated most of the times in the depressions. It is more common in the preceding associations in the line of normal succession where a high water table is present in midsummer.

In the individuals of the association not periodically burned, there is observed an invasion of shrubs and trees of the subsequent associations (Fig. 2). Among these invaders, the most important are *Aronia melanocarpa*, *Nemopanthus mucronata*, and *Picea mariana*. *Aronia melanocarpa*, besides being a destructive element of the association when it attains large size, may be considered a constituent and even a characteristic element when it occurs as seedlings of high frequency.

Species-Area Relationship

Jaccard (27) was the first to express clearly the fact that there is a relationship between the number of species and the area occupied by them. The idea was reapplied by Arrhenius in 1920 (1) who is given credit for priority by most authors because Jaccard expressed the relationship in terms of ecological variety, associated with larger areas, rather than by area per se. Jaccard said, "la richesse florale d'un territoire donné est directement proportionnelle à la diversité de ses conditions écologiques."

Arrhenius (1, 2, 3) proposed the following formula:

$$\frac{X}{X_1} = \frac{S}{s_1}^{n}$$

in which X is the area occupied by S species, X_1 the area occupied by s_1 species, and n a constant. The curve is given by using a coordinate system in which the axis of the abscissa is the logarithm of the areas and the axis of the ordinates is the logarithm of the number of species. The logarithms of the obtained points are in a straight line. In fact, the formula of such a parabola is:

$$y = x^{1/n}.$$

If one applies logarithms, there is obtained:

$$\log y = \frac{1}{n} \log x,$$

which is the formula of a straight line, where $1/n$ is the tangent of the inclination angle. If we plot the statistical data in a system of logarithmic

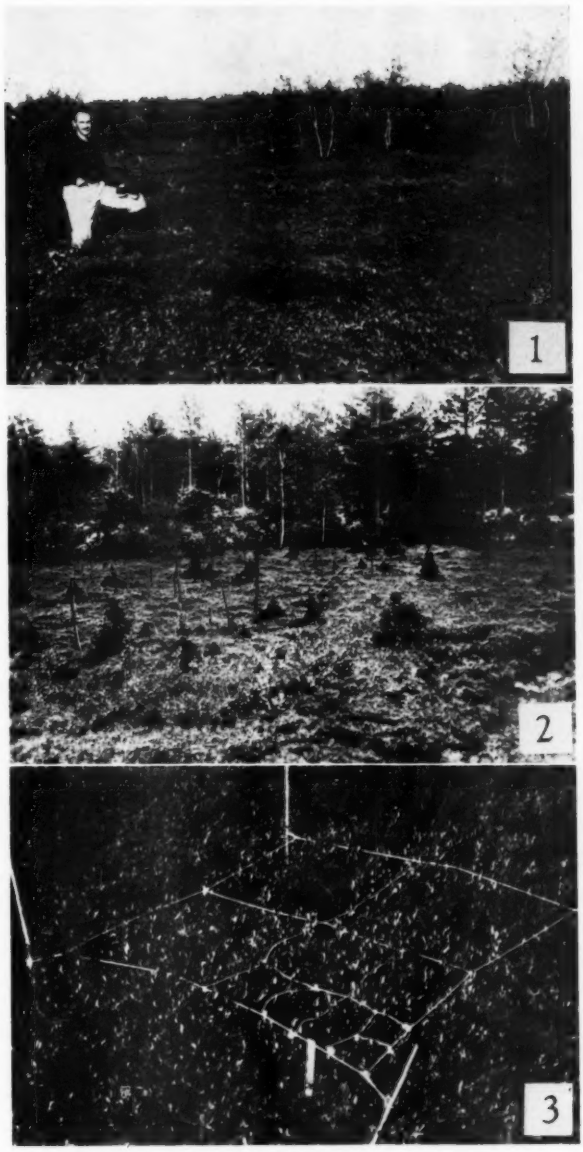

FIG. 1. Large stand of *Chamaedaphne calyculata* at Farnham, Que.
FIG. 2. First step of the invasion of a *Chamaedaphne* stand by *Picea mariana*.
FIG. 3. Frame used for field work.

PLATE I

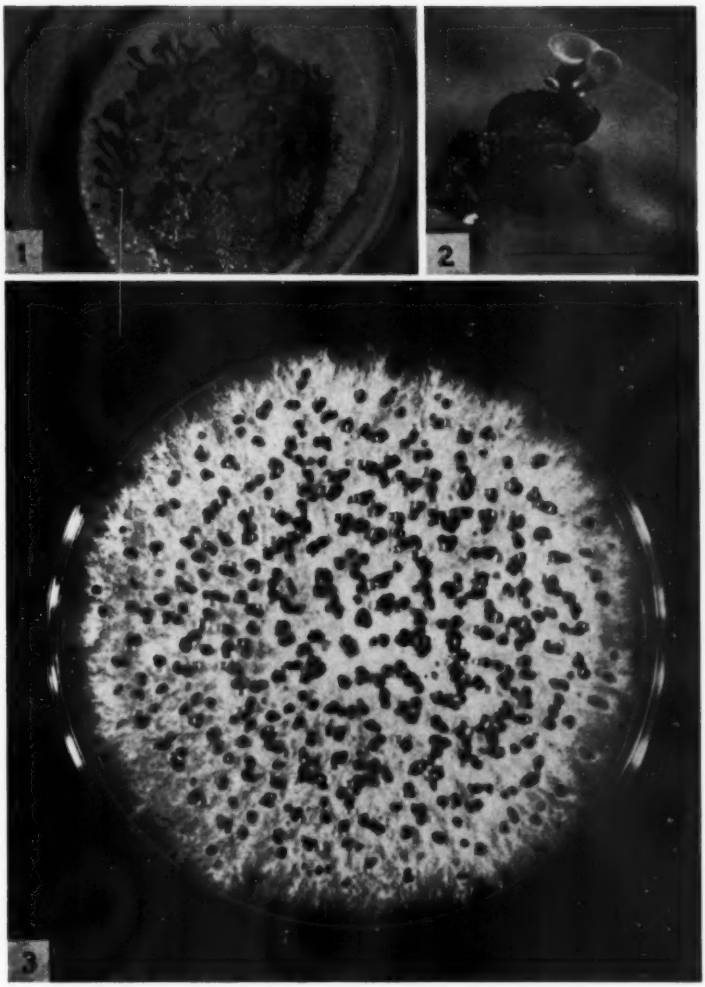

FIG. 1. Apothecia of *S. borealis* developed in culture on sand in a culture dish.
FIG. 2. Cluster of apothecia removed from the dish.
FIG. 3. Culture of *S. borealis* on potato dextrose agar grown at 10° C., five weeks old.

coordinates and obtain a straight line, we prove that the relationship between the number of species and the area follows a parabolic law.

Gleason (23, 24), criticizing Arrhenius' paper, affirms that this relationship is only true for small areas and gives absurd data for large areas.

The value of Arrhenius' law was contested by Du Rietz (18, 19, after Frey (20)) who showed that the value of the constant n varies for each association. Instead of the value 3.2 for the constant n given by Arrhenius, he found such values as 4.5 and 6.6. This does not prove that the relationship does not follow a parabolic law, but only that the straight lines, in a system of logarithmic coordinates, are more or less inclined (Frey (20)).

There are, however, curves (obtained in a system of arithmetic coordinates) that do not follow Arrhenius' formula, as they do not give straight lines in a system of logarithmic coordinates, but are concave or convex curves in relation to the axis of the ordinates. These curves are called saturation curves (Frey (20)) and are most commonly found when there is a rapid augmentation of the number of species in the areas first examined, which presently decrease to become parallel to the axis of the abscissa.

Frey (20) gives the formula

$$y = 1 - e^{-kx}$$

which is the formula of the saturation curve where the asymptote is situated at a distance 1 of the axis of the abscissa. In this formula y is the number of species in a variable surface x, e the basis of the natural logarithms and k a constant. If we introduce m as a second constant to vary the distance of the asymptote in relation to the abscissa, we find the same formula that Kylin (29, after Frey (20)) found at the time of his theoretic researches. In Kylin's formula, m is the maximum number of species in the association to be studied and k a constant, based on the assumption that all the species have the same frequency, which is not so (Frey (20)). At this point Kylin was forced to vary k in order to be closer to the truth. Kylin's formula in a system of logarithmic coordinates gives a convex curve in relation to the axis of the ordinates (Frey (20)).

Arrhenius' formula, as well as Kylin's, is only applicable to limited surfaces for, when used in very large areas, they yield, respectively, a very large number and a very small number of species.

Romell (38, 39), using a calculation of the probability of combinations of different ecological factors, found a more or less linear relationship between the number of species and the logarithm of the area. This relationship is explained in an empirical formula

$$y' - y'' = c\ (\log x' - \log x'')$$

in which y' is the number of species on the surface x', y'' the number of species in the area x'', and c a constant. The curve given by this formula is applicable in a system where the axis of the ordinates has an arithmetical scale and the axis of the abscissa has a logarithmic scale. This is called the system of semilogarithmic coordinates.

Gleason (23), in his criticism of Arrhenius' paper, found the same relationship as Romell for the communities of aspen in Michigan.

This analysis of Arrhenius's, Kylin's, and Romell's papers shows that the species–area relationship varies in the several associations for it is possible to find concrete examples of the three types of curves with the same methods of research. It seems that each author has described as general something that is real but specific.

Minimal Area

An analysis of the curves that express a relationship between species and area allowed authors such as Du Rietz and Braun-Blanquet to create the concept of minimal area. The definition of this concept varies with the several phytosociological schools. According to Du Rietz, the examined area must be sufficiently large to contain the assemblage of "constants" of the association. By constants Du Rietz understands the plants present in 90 to 100% of the stands examined, each stand being sampled by only one quadrat. The smallest area that contains the assemblage is called minimiareal by Du Rietz. According to Ruebel (41) "the minimiareal is a measure of the average individual distance of the constants of the community which stand farthest from one another" (see Cain (9)).

According to Braun-Blanquet (6, p. 52), minimal area is "the smallest area which can contain an adequate representation of an association" and furthermore "minima of space and number of species are certainly requirements which must be assigned to an association." Cain (10) gives an explanation of the "characteristic combination of species" of Braun-Blanquet, which would mean for some plant sociologists (1) the characteristic coverage of the "dominant species"; for others (2) the characteristic homogeneity of structure as shown by the "constant species", described in terms of presence, constance, and/or frequence; or (3) the presence of "characteristic species," i.e. those of high fidelity.

There are two methods for the determination of minimal area and they are called "single-plot method," and "multiple-plot method."

To determine the minimal area by the first method, one takes a quadrat and makes a list of the species which occur in this quadrat; then, another quadrat is taken, whose area will be double the first one and will include it, and to the first list any new species are added. Successive quadrats are taken whose areas are always larger than the previous quadrats and include them, until much of the surface of the stand has been covered. With the data obtained, a curve is drawn and the point that gives the minimal area is obtained by inspection and corresponds to the point where the curve approaches an asymptote to the abscissa. This process of determination fails, as was demonstrated by Cain (10), for the shape of the curve depends upon the relationship between the axes. In order to avoid this mistake, he proposes the determination of three arbitrary points, called *a*, *b*, *c* which correspond, respectively, to the points of the curve that represent (*a*) an augmentation of

10% of the total number of species with an augmentation of 10% of the total area examined, (*b*) an augmentation of 5% of the species with 10% of the total area, and, (*c*) an augmentation of 2.5% of the species with 10% of the area. At the same time that Cain proposes the three arbitrary points, he gives a mechanical process for its determination. The method consists in taking a right angle triangle and making its hypotenuse pass through the origin of the coordinates and the point which corresponds to an augmentation of 10% of the total number of species and an augmentation of 10% of the total area examined. After this, a ruler is placed beside one of the sides and the triangle is moved until its hypotenuse becomes tangent to the curve. The point of tangency is the point *a*. The other points are obtained by the same process, only with the difference that the hypotenuse of the square must pass through the origin of the coordinates and the points which correspond, respectively, to an augmentation of 5 and 2.5% of the total number of species, to an augmentation of 10% of the total area examined.

The process of the multiple-plot quadrat, as defined by Cain (11), consists in disposing on the ground a series of identical quadrats of small dimensions. Each of these quadrats is divided into smaller quadrats of different dimensions or, what is more common, in quadrats whose areas increase in geometrical progression, including or not including the preceding areas. A complete list of species is made for the first of these small quadrats to which is added the species found in subsequent quadrats. One follows this procedure for each of the quadrat series until many have been studied. After tabulation of the data the average number of species is calculated for each of the sizes of quadrats and these values are plotted in a system of coordinates, in which the axis of the abscissa represents the average number of species and the axis of the ordinates the value of the areas. Once the curve is done, the points *a*, *b*, *c* are determined by Cain's method, as it was described above. *These points represent the smallest type of quadrats that may be used, and not the minimal area of the association.* It is also necessary, therefore, to determine the smallest number of these quadrats to obtain a satisfactory sample of the association. To obtain this number, a second curve is made, but, this time, the number of quadrats is on the axis of the abscissa, and on the axis of the ordinates the number of species which are obtained when 1, 2, 3 . . . *n* quadrats of the type determined by the first curve are taken. The points *a*, *b*, *c* obtained by the method referred to above, will give the minimal number of quadrats necessary. The utility of this method is that it indicates the least size and the minimal number of quadrats that must be used in quantitative sociological studies. It is possible also to consider this as a type of minimal area.

In order to determine the minimal area of the *Chamaedaphne* association by the single-plot method, there were used nine different sizes of quadrats, with areas respectively of 1, 2, 4, 8, 16, 32, 64, 128, and 256 square meters, disposed in a fan-like arrangement, i.e., diverging from an initial point in a single direction, as illustrated in Fig. 4.

In Farnham bog two different measures were taken and, in Lanoraie, four different ones.

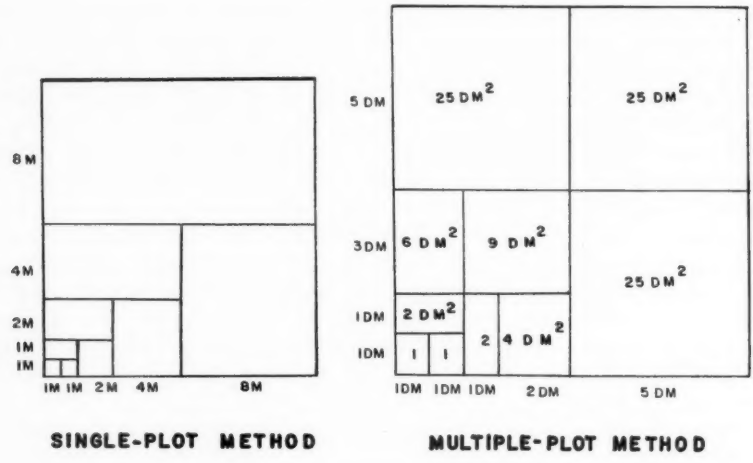

FIG. 4. Arrangements of quadrat sets.

In Table I we see that, for one of the sets in Farnham, the total of species was 25, while in another set it was only 19. In the four sets of Lanoraie the total number of species was the same, but it was obtained on different areas. In the two first sets a total of 13 was obtained on an area of 256 square meters, while in the third set this total was reached on an area of only 16 square meters, although the search was extended to an area of 256 square meters, as it was on the other sets, but no new species were encountered. For the fourth, the total number of species was reached also on a relatively small area, i.e., on an area of 64 square meters. These different results show that to have an idea of the true minimal area it may be necessary to do several

TABLE I

SPECIES–AREA CURVE DATA OBTAINED BY THE SINGLE-PLOT METHOD

	Area in m.²								
	1	2	4	8	16	32	64	128	256
Lanoraie—1	9	9	10	10	10	11	11	11	13
Lanoraie—2	10	10	10	10	11	11	11	11	13
Lanoraie—3	6	9	9	10	13	13	13	13	13
Lanoraie—4	6	6	6	7	9	11	13	13	13
Average	7.75	8.5	8.75	9.25	10.75	11.5	12.0	12.0	13.0
Farnham—1	9	9	9	10	13	15	17	20	25
Farnham—2	6	7	7	9	12	15	17	18	19
Average	7.5	8	8	9.5	12.5	15	17	19	22

determinations in the same station. In order to eliminate the error of taking empirically one of the determinations as the most characteristic, we traced curves using the averages of the different determinations for each station. Both curves were traced on a system of coordinates with a ratio of 1 : 2, in order to facilitate the comparison.

For the *Chamaedaphne* station situated at Lanoraie, we obtained a curve (Fig. 5) which gave the points: *a*, 22 sq. m.; *b*, 28 sq. m.; *c*, 38 sq. m. I chose as minimal area the point *b* to give a safe margin.

For the station situated at Farnham, the curve (Fig. 5) gave points completely different from the ones obtained for Lanoraie, specifically: *a*, 35

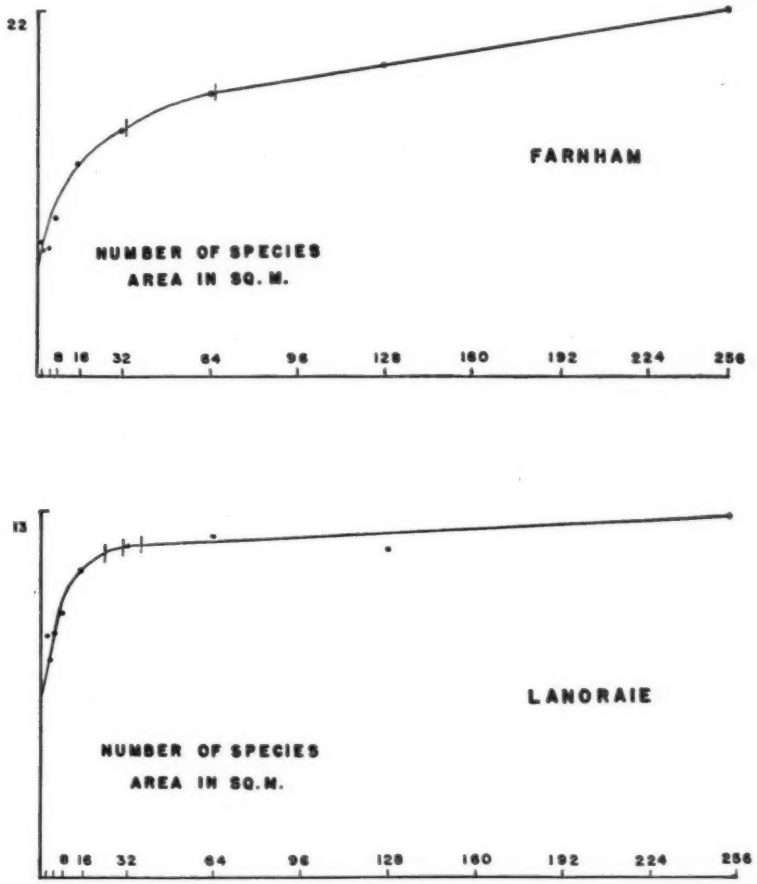

FIG. 5. Minimal area of the *Chamaedaphne* community, single-plot method.

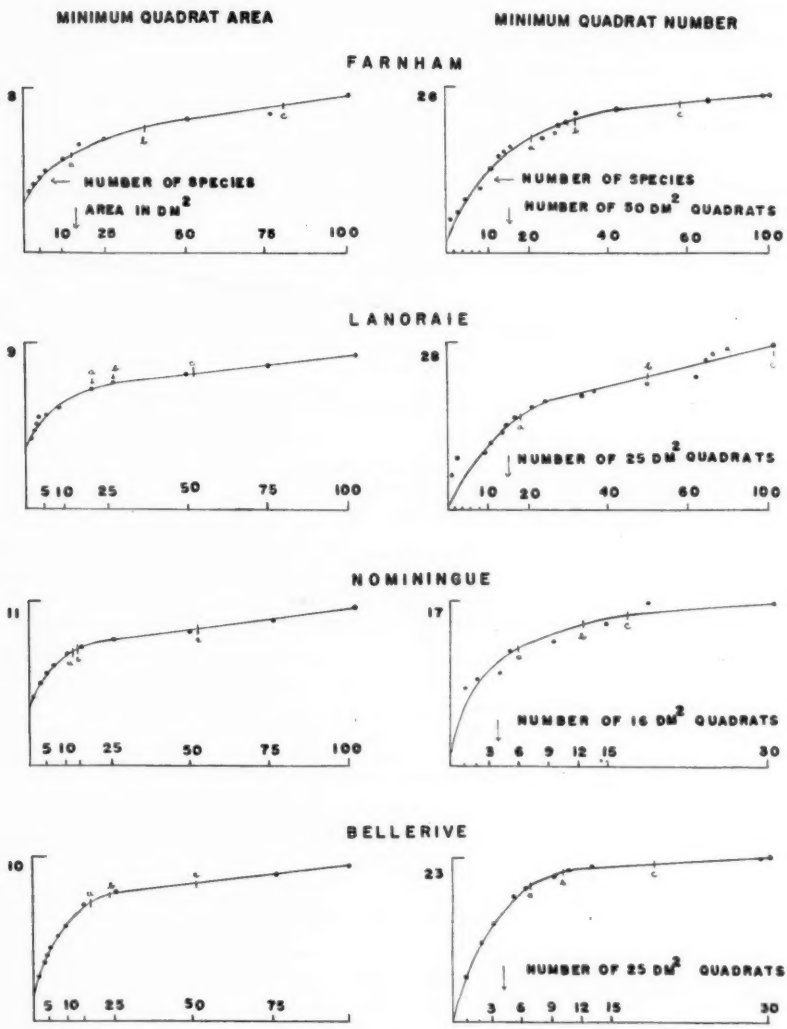

FIG. 6. Minimal area of the *Chamaedaphne* community, multiple-plot method.

sq. m.; *b*, 67 sq. m. The point *c* was not obtained, as the tangency point for this relation falls outside the limits of the graph. We chose for minimal area point *b* for the same reason as for Lanoraie.

Comparing the results obtained, we verified that the value of point *a* for Farnham is little less than the value of point *c* for Lanoraie, and that the minimal area for Lanoraie is smaller than half of the minimal area for Farnham. Because of this we did not trace a single curve for the association utilizing the averages, as we did for each of the stations. We preferred to consider the minimal area as different for each of the stations, as only a great number of determinations done in equal number of stations would allow us to have an idea of the true minimal area of the association.

Both the curves are parabolic, for when plotted on logarithmic coordinates (Figs. 7 and 8), they give a straight line.

In the determination of the minimal area by the multiple-plot process, there were laid out, in each of the stations, three sets, each one composed of 30 quadrats about 1 meter apart, disposed in a "T" shape, each branch having 10 quadrats. Each of the quadrats was divided into 10 smaller ones (Figs. 3 and 4) with areas, respectively, of 1, 1, 2, 2, 4, 6, 9, 25, 25, and 25 square decimeters. For each of these quadrats was compiled a complete list of the species present, there being about 900 different lists for each of the stations. By means of different combinations of small quadrats, one may obtain any area wished, by counting in the lists only the species present in the quadrats under consideration and being careful to eliminate the repetitions. To trace the curves it was decided to use only the areas of 1, 2, 2, 4, 5, 6, 10, 16, 25, 50, 75, and 100 sq. dm. All the curves were traced in a system of coordinates whose relation between the axes of *y* and *x* is of 1 to 2. The points *a*, *b*, *c*, were determined as before.

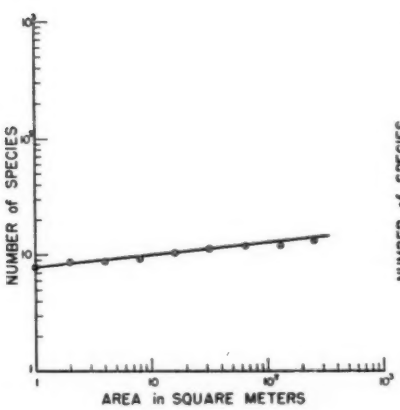

FIG. 7. Straight line obtained with the minimal area data (single-plot method) for Lanoraie.

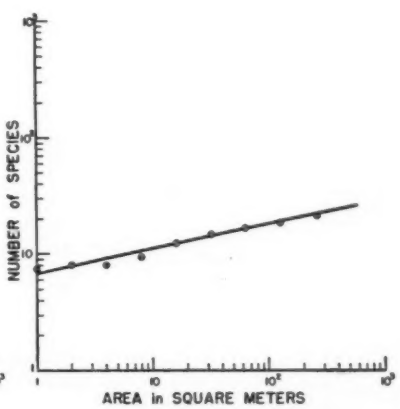

FIG. 8. Straight line obtained with the minimal area data (single-plot method) for Farnham.

Once the minimal quadrat was determined, a curve was plotted to obtain the minimal number of quadrats necessary to give a suitable representation of the association. The scale used was the same as for the determination of the quadrat area.

For Farnham bog, the curve (Fig. 6) which gave the area of quadrat was based only on 80 quadrats, the other 10 having been eliminated because they were not taken inside the association. The points obtained were, respectively, *a*, 14 sq. dm.; *b*, 37.5 sq. dm.; *c*, 80 sq. dm. In the determination of the minimal number of quadrats, we used, to facilitate the calculation, an area of 50 square decimeters, not only because it would be difficult to get areas of 37.5 sq. dm., but to facilitate the field work. The curve for the minimal number of quadrats (Fig. 6), gave the points *a*, 20; *b*, 31; *c*, 58. From the results obtained, we deduced that, to get a suitable representation of the *Chamaedaphne* association in Farnham bog, it is necessary to dispose in a "T" shape about 30 quadrats, each with an area of 50 sq. dm., which gives a total sampled area of 15 square meters. Comparing this result with the one obtained by the single-plot method (Table II), we see that this is a much smaller one than the other which is to be expected from the scattering of the plots.

In the bogs at Bellerive and Nomininge, because of the difficulties of access, only 30 quadrats were done in each of these bogs, instead of 90, as was planned. In Bellerive, the curves (Fig. 6) gave us *a*, 16.5 sq. dm.; *b*, 22.5 sq. dm.; *c*, 50 sq. dm.; for numbers *a*, 6; *b*, 10; *c*, 16. In the second curve, to facilitate the calculation, the area used was the one of 25 square decimeters, instead of 22.5 square decimeters. The minimal sampled area for this station is 2.5 square meters, or 10 quadrats with an area of 25 square decimeters each.

In Nomininge bog, the results obtained by the curves (Fig. 6) were for area: *a*, 12.5 sq. dm.; *b*, 15 sq. dm.; *c*, 52.5 sq. dm.; for number: *a*, 6; *b*, 13; *c*, 17. The minimal area sampled for this station is about 2 square meters, or 13 quadrats with an area of 16 square decimeters each.

TABLE II

COMPARISON OF THE RESULTS OBTAINED FOR THE MINIMAL AREA OF THE *Chamaedaphne* COMMUNITY

	Multiple-plot method						Single-plot method		
	Minimum quadrat area in $dm.^2$			Minimum quadrat number			Minimum quadrat area in $m.^2$		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Lanoraie	16	25	52	18	50	80	22	28	38
Farnham	14	37.5	80	20	31	58	35	67	
Bellerive	16.5	22.5	50	6	10	16			
Nomininge	12.5	15	52.5	6	13	17			

TABLE III

PHYTOSOCIOLOGICAL TABLE

Number of the station	1	2	3	4	5	6	7	8	9	10	11	Constancy		
Area of the quadrat in m.²	100	100	100	60	100	100	25	25	100	100	100			
Cover of shrub layer in %	70	90	60	60	40	90	75	60	100	70	40			
Cover of herb-moss layer in %	100	10	80	85	80	35	1	80	100	100	90	%	Class	L.F.
Shrub layer:														
Chamaedaphne calyculata	3.5	4.5	4.4	4.4	3.4	4.4	4.4	3.4	3.3	3.4	3.5	100	V	N
Kalmia angustifolia	+ +	. .	. .	+ +	+ +	1.1	2.2	1.1	1.1	1.2	+ +	100	V	N
Ledum groenlandicum	2.4	. +	+ +	1.3	—	+ 1	+ +	+ +	+ +	+ 1	2.2	99.9	V	N
Kalmia polifolia	. .	. .	+ +	1.1	—	+ +	—	—	1.1	. .	+ +	72.7	IV	N
Andromeda glaucophylla	. .	+ +	1.1	. .	—	. .	—	—	2.2	+ +	. .	72.7	IV	N
Vaccinium angustifolium	1.1	—	+ 1	—	—	3.3	—	—	—	+ 1	+ +	45.4	III	N
Myrica gale	—	+ 1	—	. .	—	—	—	—	2.2	—	—	27.2	II	N
Vaccinium canadensis	—	—	—	—	—	—	—	—	—	—	+ +	9.09	I	N
Vaccinium sp.	—	—	—	—	+ +	—	—	—	—	—	—	9.09	I	N
Rhododendron canadense	—	—	—	—	—	—	—	—	—	—	+ +	9.09	I	N
Aronia melanocarpa	—	—	—	—	—	—	—	—	—	+ +	—	9.09	I	M
Nemopanthus mucronata	—	—	—	—	—	—	—	—	—	. .	—	9.09	I	M
Rubus chamaemorus	—	—	—	—	—	—	—	—	+ +	—	—	9.09	I	Ch
Viburnum cassinoides	—	—	—	—	+ +	—	—	—	—	—	—	9.09	I	M
Spiraea latifolia	—	—	—	—	—	—	—	—	—	. .	—	9.09	I	N
Herbaceous layer:														
Carex spp.	1.1	—	—	—	+ +	—	—	—	+ +	. .	3.4	45.4	III	H
Vaccinium oxycoccos	+ +	. .	2.2	—	—	. .	—	—	—	—	1.4	45.4	III	Ch
Smilacina trifolia	—	—	1.1	—	—	—	—	—	1.1	. .	—	27.2	II	G
Carex trisperma	—	—	+ 1	—	—	—	—	—	—	—	—	9.09	I	H
Eriophorum spissum	—	—	—	. .	—	—	—	—	—	—	1.2	18.1	I	H
Eriophorum angustifolium	—	—	—	—	—	—	—	—	—	—	. .	9.09	I	H
Eriophorum sp.	—	—	—	—	—	. +	—	—	—	—	—	9.09	I	H
Scirpus sp.	—	—	—	—	—	—	+ +	—	—	—	—	9.09	I	G
Calla palustris	—	—	—	—	+ +	—	—	—	+ 2	—	—	18.1	I	HH
Sarracenia purpurea	—	—	—	—	—	. .	—	. .	—	—	—	18.1	I	H
Moss layer:														
Sphagnum spp.	5.5	1.2	4.5	3.5	4.5	1.2	—	+ 3	5.5	5.5	4.5	99.9	V	
Polytrichum strictum	1.1	—	—	2.3	+ 2	—	—	4.5	—	1.1	1.2	53.6	III	
Aulacomnium palustre	—	—	—	—	—	—	—	1.5	—	+ 2	—	18.1	I	
Dicranum sp.	—	—	—	. .	—	—	—	—	—	—	—	9.09	I	
Pleurozium schreberi	—	—	—	1.5	—	+ 1	—	—	—	—	—	18.1	I	
Moss	—	—	—	—	—	—	2.5	—	—	—	—	9.09	I	
Cladonia rangiferina	—	—	—	. .	—	—	—	—	—	—	—	9.09	I	
Invaders:														
Picea mariana	+ +	—	—	+ +	+ +	+ +	—	—	+ +	—	—	45.4	III	M
Larix laricina	—	—	—	1.1	+ +	—	—	—	+ +	—	—	27.2	II	M
Pinus strobus	—	—	—	—	. .	. .	—	—	—	—	—	18.1	I	M
Betula populifolia	—	—	—	—	. .	—	—	—	—	. .	—	18.1	I	M
Alnus rugosa	+ +	—	—	—	—	—	—	—	—	—	—	9.09	I	M
Acer rubrum	—	—	—	—	+ +	—	—	—	—	—	—	9.09	I	M
Salix discolor	—	—	—	—	+ +	—	—	—	—	—	—	9.09	I	M
Salix sp.	—	—	—	—	+ +	—	—	—	—	—	—	9.09	I	M

No. of vascular species, 33 = 11M + 11N + 2CH + 6H + 2C + 1HH.
No. of mosses and lichens, 7.

Comparing the results obtained for the different stations (Table II), we see that the minimal area of the association varies for each of the stations and also with the number of quadrats taken. As a matter of fact, examining the table referred to above, we see that the stations of Bellerive and Nomininge, in which was taken the same number of quadrats, show similar results; the same happening with Lanoraie and Farnham (88 and 80 quadrats, respectively). As we verified above, the minimal areas obtained by the two methods differ a great deal for the same station and this shows that the results obtained for a minimal area are dependent also on the method utilized and the disposition of quadrats.

This study can not give the minimal area for the *Chamaedaphne* association, as there are lacking sufficient data to obtain a suitable average.

Cover and Sociability of the Species

Phytosociological surveys, including cover and sociability for each of the species, were done at 11 different stations. For each of these stations only one quadrat was used, generally with an area of 100 square meters. In other stations quadrats of 25 square meters were used. In both cases, they were situated in the midst of the community and their shape was a square. In order to compare the data obtained, they were organized in a table and the constancy calculated (Table III). In this table the stations were numerated from 1 to 11, corresponding to the following localities:

1. Bog 50 miles South of the village of Timagami, Timagami Provincial Forest, Sudbury Co., Ont. Irregular local topography, the cushions of *Sphagnum* reaching about 1 meter in height.

2. Bog five miles South of Lac des Loups and half a mile North of Lac Roland, Pontiac Co., Quebec. Local topography very irregular, the cushions of *Sphagnum* reaching about 50 cm. The *Chamaedaphne* community seems to have developed after trees of the association *Picea mariana – Kalmia angustifolia* were cut. The presence of stumps of trees shows that they had been spaced at least 5 meters apart.

3. Bog in the same locality as number 2, but on the opposite side of the road. Local topography very irregular, the cushions of *Sphagnum* never developing more than 30 cm. in height.

4. Bog with irregular topography, situated near the town of Kirkland Lake, Parry Sound Co., Ont.

5. Bog along the road 4 miles North of Waterloo, Shefford Co., Quebec.

6. Bog recently burned in the arborescent stage, situated just North of town of Barrys Bay, Renfrew Co., Ont.

7. Bog at Alfred, Prescott Co., Ont. This bog has an area of about 6800 acres, of which 4000 are covered by the *Chamaedaphne* community. The surface is completely flat. The *Chamaedaphne* community is burned periodically and has been destroyed in several places by the exploitation of peat.

8. Station in the same place as No. 7, but on a part of the bog less devastated and where fire has not passed recently.

9. Station at the margins of Lake Shipshaw, Chicoutimi Co., Quebec, about 140 miles North of Dolbeau, near Passes Dangereuses.

10. Bog 1 mile East of the railroad station Ste. Brigide, 3 miles West of Farnham, Iberville, and Missisquoi counties, Quebec.

11. Bog near the Lanoraie station, 40 miles East of Montreal, Berthier-Joliette counties, Quebec.

In the four bogs where the minimal area study was made (Lanoraie, Farnham, Bellerive, and Nominingue), a sociological study based on scattered 1 sq. m. plots was also made. In this way, about 88 surveys were obtained for Lanoraie, 80 for Farnham, and 30 for Bellerive and Nominingue.

To each of the species present in the quadrats, either the ones with an area of 100 square meters or the ones with an area of 1 square meter, figures were assigned for its cover and sociability. It is necessary to define the concepts of cover and sociability that have been employed.

Cover of a species is the area shaded or occupied by the individuals of the species in the quadrat under consideration (Braun-Blanquet (7), Cain (8), Oosting (31)). The cover degree of the species allows us to determine the dominance. The scale used was composed of seven classes, with the following values:

(.) – Species only present, without cover of any importance; generally seedlings.
\+ – Species with a definite cover, but less than 1%.
1 – Species covering from 1% to 20% of the area.
2 – Species covering from 21% to 40% of the area.
3 – Species covering from 41% to 60% of the area.
4 – Species covering from 61% to 80% of the area.
5 – Species covering from 81% to 100% of the area.

The cover estimate is determined by the vertical projection of the foliaceous area onto the soil.

As may be seen from the above, a combination scale of abundance and cover, as recommended by Braun-Blanquet (6), was not used so that it would be possible to make a statistical study of the data and also because abundance is a relative appreciation of the number of individuals of each species and not the exact number of individuals (Cain (8)) nor their coverage.

Sociability is the manner in which individuals of each species are disposed one in relation to another (Braun-Blanquet (7), Cain (8), Oosting (31)). It is essentially a qualitative characteristic for each of the species and, therefore, it is not possible to employ such data statistically. Each species has a sociability range, either in the same habitat or in different habitats. In our

studies, however, we use a concept of sociability different from the one used by the majority of phytosociological schools. The determination of sociability consists in a new cover estimate for each part of the quadrat in which there is a larger concentration of individuals of the species. This procedure is proposed by Dansereau (15) and it seems to me that this method expresses not the sociability of the species but only the *local abundance*, which is useful in the recognition of the aggregations of the individuals of each different species in the quadrat. In this paper, we shall, however, apply the term of sociability to local abundance.

Analyzing Table III, it is found that *Chamaedaphne calyculata* maintains approximately the same cover in the 11 stations; this varies between classes 3 and 4, the same being true of sociability. *Kalmia angustifolia* always presented a low cover, never surpassing class 1 except in survey No. 7, in which it was codominant. *Ledum groenlandicum*, in six of the surveys, presented a cover of less than 1%, being even absent from survey No. 5; in contrast, in surveys 1 and 11 it was codominant. *Kalmia polifolia* always occurred with less than 1%, being absent in three of the surveys. The same behavior is found in *Andromeda glaucophylla*, which is codominant together with *Myrica gale*, in survey No. 9, but of low cover or absent in the other stations. *Vaccinium angustifolium* rarely shows a high degree of cover and, in the 11 surveys, was codominant only in No. 6, with a cover of class 3. *Rhodora canadensis*, *Aronia melanocarpa*, *Nemopanthus mucronata*, *Rubus chamaemorus*, *Viburnum cassinoides*, *Spiraea latifolia* were found in only one survey each and then with a very low coverage.

In the inferior synusia, *Sphagnum* is dominant, presenting in general high cover and sociability; but in certain stations these values decrease, as in surveys 2, 6, 7, and 8. *Polytrichum strictum*, when present, has a cover whose value is situated in the middle classes. Besides *Sphagnum*, the most conspicuous species of this layer is, without any doubt, *Vaccinium oxycoccos*, which has however a relatively low cover, generally less than 1%.

Of the invaders, i.e., plants belonging to subsequent associations in the normal succession, only *Picea mariana* presents a great number of individuals, but it nevertheless has a very low cover. Observing the cover and sociability degrees of the species in Table III, we see that an area of 100 square meters is so large for this type of vegetation that it is difficult to calculate the cover degrees, so that one gets a false impression of the importance of each species.

A table of distribution by cover classes and sociability was organized for each of the four stations studied in detail (Tables IV, V, VI, and VII). The construction of these tables facilitates not only the calculation of average cover, but it also decreases the necessity of publishing a large sociological tabulation. In these tables the species are arranged alphabetically within decreasing cover classes. Mosses and lichens are placed apart.

For the calculation of the average cover, to each of the cover classes was attributed a median value and (.), +, and 1 were considered as a single class,

TABLE IV

DISTRIBUTION IN COVERAGE AND SOCIABILITY CLASSES OF THE SPECIES OF THE *Chamaedaphne* COMMUNITY AT LANORAIE, QUEBEC PROVINCE

		Cover									Sociability							
	L.F.	(·)	+	1	2	3	4	5	N	x%	Class	(·)	+	1	2	3	4	5
Chamaedaphne calyculata	N	2	6	17	22	25	13	2	87	37.3	2	2	4	8	20	17	26	10
Alnus incana	M	1							1	10	1	1						
Andromeda glaucophylla	N	9	2						11	10	1	7	4					
Andropogon sp.	H	1							1	10	1	1						
Aronia melanocarpa	M	4	2						6	10	1	4	2					
Betula populifolia	M	1		1					2	10	1	1				1		
Carex disperma	H	5	2						7	10	1	4	1			1	1	
Carex trisperma	H	5	1	2	3				11	15.4	1	5		2	3	1		
Carex spp.	H	26	13	9	5	1			54	12.5	1	26	10	11	4	3		
Eriophorum angustifolium	H	2	2						4	10	1	2		2				
Eriophorum spissum	H	11	15	12	4				42	11.9	1	5	7	16	9	5		
Eriophorum virginicum	H	1							1	10	1	1						
Eriophorum sp.	H	4	4	4	2				14	12.8	1	4	3	5	1		1	
Glyceria sp.	H	1							1	10	1	1						
Kalmia angustifolia	N	12	21	9	3	1	2		48	14.5	1	10	18	8	8	2	1	1
Kalmia polifolia	N	35	26	1					62	10	1	34	23	5				
Ledum groenlandicum	N	14	16	25	10		1		66	13.9	1	14	13	12	21	5	1	
Nemopanthus mucronata	M	3	1						4	10	1	3	1					
Populus tremuloides	M	1							1	10	1	1						
Rhododendron canadense	N	16	10	2	1				29	10.6	1	14	11	3	1			
Sarracenia purpurea	H	5							5	10	1	5						
Spiraea latifolia	N	1							1	10	1	1						
Spiraea tomentosa	N	1							1	10	1	1						
Vaccinium angustifolium	N	30	14	6					50	10	1	29	10	10	1			
Vaccinium canadensis	N	7	4						11	10	1	7	1	3				
Vaccinium oxycoccos	Ch	20	10	5	2				37	11	1	19	9	6	1	1	1	
Mosses and lichens:																		
Sphagnum spp.			3	3	11	9	18	43	87	68.6	4			2	1	5	8	71
Aulacomnium palustre		1							1	10	1	1						
Cladonia cristatella		6	1						7	10	1	6	1					
C. pyxidata var. *chlorophaea*		1							1	10	1	1						
Polytrichum strictum		16	28	27	9	7			87	15.2	1	15	22	18	10	13	7	2

TABLE V

DISTRIBUTION IN COVERAGE AND SOCIABILITY CLASSES OF THE SPECIES OF THE *Chamaedaphne* COMMUNITY AT FARNHAM, PROVINCE OF QUEBEC

		Cover									Sociability							
	L.F.	(.)	+	1	2	3	4	5	N	x%	Class	(.)	+	1	2	3	4	5
Chamaedaphne calyculata	N	1	1	16	25	19	12	4	78	39.4	2	1		8	25	20	14	10
Andromeda glaucophylla	N	5	1	2					8	10	1	5	1	1	1			
Aronia melanocarpa	M	41	17	1					59	10	1	40	17	2				
Betula populifolia	M	23	1						24	10	1	23		1				
Carex trisperma	H	2							2	10	1	1	1					
Carex spp.	H	20	4	1					25	10	1	19	4	2				
Chiogenes hispidula	Ch	1							1	10	1	1						
Eriophorum spissum	H	1	1						2	10	1	1	1					
Kalmia angustifolia	N	22	18	18	1				59	10.3	1	17	15	11	14	2		
Kalmia polifolia	N	24	7						31	10	1	24	6	1				
Ledum groenlandicum	N	17	6	7	2	1			33	12.4	1	13	6	4	6	2	2	
Nemopanthus mucronata	M	10	2						12	10	1	10		2				
Rhododendron canadensis	N	8	4						12	10	1	6	5	1				
Senecio sp.	H	1							1	10	1	1						
Smilacina trifolia	G	5							5	10	1	5						
Spiraea latifolia	N	14	1						15	10	1	14	1					
Spiraea tomentosa	N	2							2	10	1	2						
Vaccinium angustifolium	N	11	7	1					19	10	1	8	9	2				
Vaccinium canadensis	N	7	1						8	10	1	7	1					
Vaccinium oxycoccos	Ch	3							3	10	1	3						
Mosses and lichens:																		
Sphagnum spp.			1	2	1	4	13	57	78	80.7	4		1			3	10	64
Aulacomnium palustre		14	2	3					19	10	1	9	5	2		1		2
Cladonia pyxidata		2							2	10	1	2						
C. pyxidata var. *chlorophaea*		11							11	10	1	11						
Cladonia sp.		1							1	10	1	1						
Polytrichum commune		8	7	6	2				23	11.7	1	4	9	1	2	3	1	3
Polytrichum strictum		16	30	21	7	1			75	12.4	1	14	25	18	6	5	2	5

No. of vascular species, 20 = 3M + 10N + 2Ch + 4H + 1G.
No. of mosses and lichens, 7.

TABLE VI

DISTRIBUTION IN COVERAGE AND SOCIABILITY CLASSES OF THE SPECIES OF THE *Chamaedaphne* COMMUNITY AT NOMININGUE, QUEBEC PROVINCE

		Cover									Sociability							
	L.F.	(.)	+	1	2	3	4	5	N	x%	Class	(.)	+	1	2	3	4	5
Chamaedaphne calyculata	N		1	3	6	12	6	2	30	47.33	3			1	5	8	13	3
Ledum groenlandicum	N	1	5	10	8	5	1		30	24	2	1	4	8	6	5	5	1
Andromeda glaucophylla	N	4							4	10	1	4						
Andropogon sp.	H	1	1						2	10	1	1		1				
Eriophorum spissum	H	11	3	1					15	10	1	9	3	2	1			
Carex spp.	H	12	3	1					16	10	1	12	2	1	1			
Kalmia angustifolia	N	5	16	7	2				30	11.33	1	4	14	7	3	2		
Kalmia polifolia	N	20	8						28	10	1	20	7	1				
Larix laricina	M	1							1	10	1	1						
Nemopanthus mucronata	M	6							6	10	1	6						
Picea mariana	M	3							3	10	1	3						
Smilacina trifolia	G	2							2	10	1	2						
Vaccinium canadensis	N	21	2						23	10	1	21	1	1				
Vaccinium oxycoccos	Ch	11	9	2	2				24	11.6	1	11	5	4	3	1		
Viburnum cassinoides	M	1							1	10	1	1						
Mosses and lichens:																		
Sphagnum spp.					1	2	3	24	30	83.33	5							30
Aulacomnium palustre		8	2						10	10	1	5	3	2				
Pleurozium schreberi		5	3						8	10	1	2	4	1	1			
Pohlia nutans		2		1					3	10	1	2						
Polytrichum strictum		8	13	4	2	1			28	12.8	1	8	12	3	1	2		1

No. of vascular species, 15 = 4M + 6N + 1Ch + 3H + 1G.
No. of mosses and lichens, 5.

TABLE VII

DISTRIBUTION IN COVERAGE AND SOCIABILITY CLASSES OF THE SPECIES OF THE *Chamaedaphne* COMMUNITY AT BELLERIVE, QUEBEC PROVINCE

	L.F.	Cover									Sociability							
		(.)	+	1	2	3	4	5	N	x%	Class	(.)	+	1	2	3	4	5
Chamaedaphne calyculata	N				2	5	14	9	30	70	4					2	15	13
Ledum groenlandicum	N		5	16	5	1	2	1	30	21.3	2		4	10	11	1	3	1
Vaccinium oxycoccos	Ch	3	2		1		1		7	21.4	2	2	3				2	
Abies balsamea	M	1							1	10	1	1						
Andromeda glaucophylla	N	7	4						11	10	1	7	3	1				
Carex disperma	H	1							1	10	1	1						
Carex trisperma	H	6							6	10	1	5	1					
Gaultheria procumbens	Ch		3						3	10	1		3					
Kalmia angustifolia	N	3	20	5	1				29	10.6	1	3	19	4	2	1		
Kalmia polifolia	N	6	1						7	10	1	6	1					
Larix laricina	M	6							6	10	1	6						
Scirpus sp.	G	5	3	5		1			14	12.8	1	4	2	5	2	1		
Vaccinium angustifolium	N		1						1	10	1		1					
Vaccinium canadensis	N		15	6	7	1			29	16.3	1		14	5	7	2	1	
Mosses and lichens:																		
Aulacomnium palustre		15	3						18	10	1	14	3					1
Cladonia pyxidata		1							1	10	1	1						
Cladonia rangiferina		1							1	10	1	1						
Hypnum crista-castrensis		2							2	10	1	1	1					
Pleurozium schreberi		4	11	4	2	1			22	13.6	1	2	6	2	4	4		4
Pohlia nutans		12							12	10	1	10	2					
Polytrichum strictum		10	3	3	2				18	12.2	1	5	6	1	1		2	3
Sphagnum spp.		6	5	3	5				19	15.5	1		4	1				14

No. of vascular species, 14 = 2M + 7N + 2Ch + 2H + 1G.
No. of mosses and lichens, 8.

for under the statistical point of view, in order to calculate averages one must use classes of the same order. The average points attributed to the classes were:

Class 1 ((.), +, and 1)	– 10%
Class 2	– 30%
Class 3	– 50%
Class 4	– 70%
Class 5	– 90%

The averages were calculated by means of the formula:

$$\overline{X} = \overline{Z} + \frac{\Sigma\,(fd')}{N}\,C$$

in which $\overline{Z}$ is guessed mean; f – frequency; d' – deviation from arbitrary origin; N – total number of cases; C – size of class interval.

The method commonly used to calculate the average cover by the addition of classes and division of the total by the number of occurrence is not right, for the classes are of great amplitude and two or more species situated in the same one do not have the same cover. It is therefore more correct to give each of the classes an average value, so that one may utilize statistical analysis. The attribution of the values 0.25 and 0.5 to the classes (.) and + would not be a correct procedure, as these two classes do not actually correspond to cover, but only to presence.

At Lanoraie (Table IV) only two species presented an average cover of a class superior to class 1, *Chamaedaphne calyculata* in class 2, and *Sphagnum* spp. in class 4. The same occurred at Farnham (Table V). At Bellerive (Table VII), among 22 species only three have classes superior to 1: *Chamaedaphne* reached class 4, while *Ledum groenlandicum* and *Vaccinium oxycoccos* reached only class 2. At Nomininguе (Table VI), among 20 species only three reached classes with relatively high cover.

We shall now compare surveys 10 and 11, of Table III, with the distribution tables of the cover classes for the stations of Farnham and Lanoraie. These surveys were made at the same locality as the 1 square meter ones. Surveys 10 and 11 were taken on an area of 100 square meters, while the ones given in Tables IV and V correspond, respectively, to 80 and 88 quadrats of 1 square meter. Comparing the results for several species, we notice, for example, that *Chamaedaphne calyculata* in surveys 10 and 11 was estimated as class 3, while in the average of the several surveys of 1 square meter it was assigned to class 2; *Ledum groenlandicum* was in classes + and 2, while in the surveys of 1 square meter it was in class 1. Other species such as *Nemopanthus mucronata*, *Rhodora canadensis*, *Aronia melanocarpa* were not even present in surveys on an area of 100 square meters, although ranking in class 1 in the surveys of 1 square meter for both stations.

From this comparison, we deduct that the use of a great number of small quadrats gives a better idea of the cover and sociability of the species and also of the presence or absence of species in the surveys. Furthermore, this

research method allows the analysis of a larger total area in better detail than the use of a single large quadrat. Another advantage of this method is that the data may be used for the determination of frequency of the species.

Frequency

Frequency is the concept that expresses the uniformity with which plants are distributed throughout the community. It is determined by a great number of quadrats of the same size, distributed all over the stand. For the determination of frequency, a list is made of all the species present in each of the quadrats. The frequency of each species is given by the relation in percentage between the number of areas which contain the species and the total number of quadrats used. After obtaining the frequency percentage, the species are classified by certain authors in a 10-class scale, or more commonly in a five-class scale:

Class A—species present in 1% to 20% of the quadrats.
Class B—species present in 21% to 40% of the quadrats.
Class C—species present in 41% to 60% of the quadrats.
Class D—species present in 61% to 80% of the quadrats.
Class E—species present in 81% to 100% of the quadrats.

Once the frequency classes of all the species are determined, it is possible to calculate the percentage of the total species in each of the classes. The percentage of the class is plotted on a system of coordinates in which the axis of the abscissa corresponds to the classes of frequency and the axis of the ordinates to the class percentages. The curves obtained by the majority of authors present a "J" shape, i.e., a curve with two maxima, corresponding, respectively, to the classes A and E. In fact, most of the frequency studies in the literature rank class A as much larger than the other classes; i.e., the number of rare species or species with low frequency is much greater than the number of common species. There is, however, a secondary peak in class E composed of the most common species. The observation of this fact gave origin to Raunkiaer's frequency law, which may be expressed by the relation: $A > B > C \leqslant D < E$. Several authors, studying this law in detail (Romell (39, 40), Frey (20), Gleason (25), Nichols (30)), came to the conclusion that it expresses nothing more than the fact that in a plant community there is always a larger number of species with few individuals than with many.

Raunkiaer (34), attempting to establish his law, studied about 8000 frequency determinations and in each set the above relation appeared. He constructed a spectrum based upon these determinations which was called the "normal spectrum," in the sense that it represents an average and around it are disposed the spectra found in different conditions and localities. His normal spectrum presents the following percentage values: A, 53, B, 14, C, 19, D, 8, E, 16.

For North American communities, Kenoyer (28) found a normal spectrum which differs from Raunkiaer's, as it presents a larger percentage of species in class A and a smaller percentage in class E. According to Kenoyer, this fact

is due to the greater richness of the American flora. His studies were based on about 1400 determinations, which gave the class percentage relation of A, 69, B, 12, C, 6, D, 4, E, 9. It is necessary to notice that Kenoyer's association concept is completely different from Raunkiaer's (35, 36) and from that of the Zürich–Montpellier School. Analyzing his work (Kenoyer (28)), we note that the example (p. 347), which is considered as a *Chamaedaphne* association, includes more than one association according to finer concepts of the association. The example is not a characteristic *Chamaedaphne* association. I draw this conclusion because he lists such species as *Menyanthes trifoliata* — 12%, *Potentilla palustris* — 32%, etc., which indicate that the area examined was a mosaic of communities; these are aquatic plants characteristic of associations that normally precede *Chamaedaphne* in succession (Dansereau and Segadas-Vianna (16)). Also, the presence of certain weedy species suggest disturbance and possible degradation of the *Chamaedaphne* community. Hence, not only the richness of the American flora, as claimed by Kenoyer, but the breadth of his association concept would lead to a normal frequency class curve with a larger class A than that found by Raunkiaer. Furthermore, the American *Chamaedaphne* association, as we found it in Quebec, is no more rich than northern European bog communities. The results obtained by Kenoyer for the *Chamaedaphne–Sphagnum* association differ greatly from the ones we obtained, which are surprisingly close to Raunkiaer's normal spectrum (Table IX and Fig. 9).

The shape of the frequency curve varies with the area of the quadrats used and it is possible to obtain curves in which one of the maxima is not present. If we take, as an example, quadrats sufficiently small so that they include a single species each, we shall obtain an exaggeration of class A. Similarly, if we take quadrats each of which includes all the species, we obtain 100% in class E. The variation of the curve's shape and of the frequency value of the species with the type of quadrat used was studied by several authors (Romell (40), Gleason (25), Cain (9)). Gleason (25) came to the conclusion that Raunkiaer's frequency law is only apparent when the chosen type of quadrat is such that it results in a curve in "J," "and that it is obscured or lost if the quadrats are either too large or too small."

Romell (40), by means of probabilities and the suppositions that "(1) every one of the species in the community is adapted to a definite site, (2) the site factors in an area or district analyzed vary from point to point according to pure chance around a mean value, (3) on the average it is just as probable that species will be adapted to the rarer combinations of site factors as to those common in the particular area," obtained theoretical curves which coincide exactly with the ones obtained empirically.

Cain (9), looking for a method to determine the type and number of quadrats necessary to obtain a normal curve, i.e., which would be close to the one found by Raunkiaer, concluded that they may be determined by his multiple-plot method. This way, his method not only gives a minimal area of the association but also the type and number of quadrats necessary to obtain the frequency indices of the species.

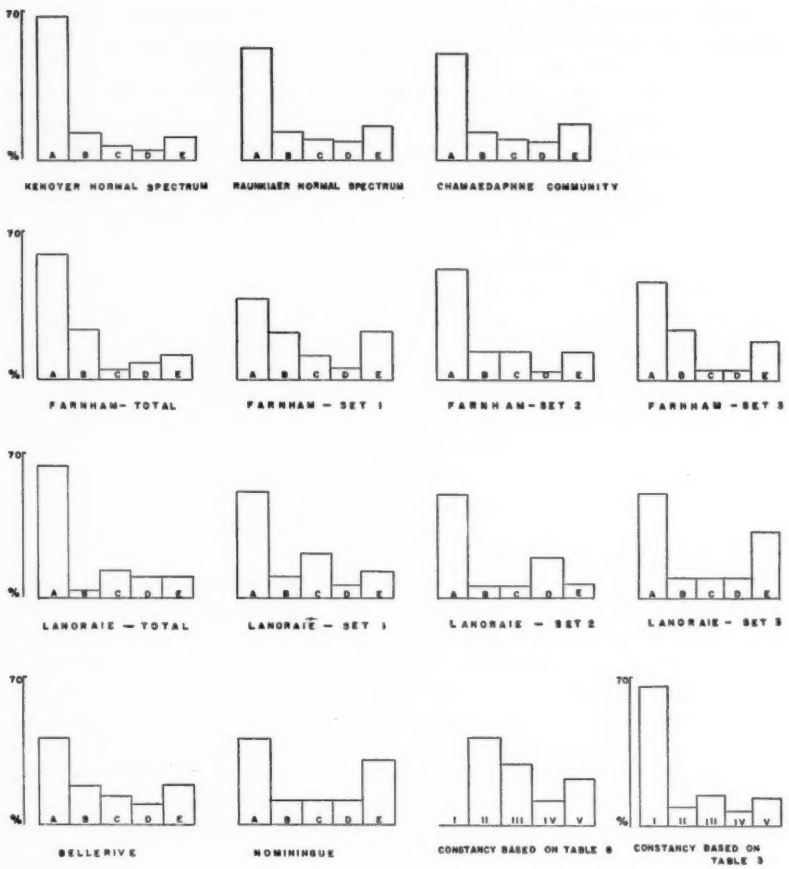

FIG. 9. Frequency and constancy class distribution compared with the "normals" of Kenoyer and Raunkiaer.

Our frequency indices were based upon the same quadrats of 1 square meter used to determine the other characteristics of this community. The results obtained for the four stations are given in Tables VIII and IX. Fig. 9 gives a comparison of the spectra for each set and station, as well as for the association, together with the normal spectra of Kenoyer and Raunkiaer.

In Table IX and Fig. 9 we see that the spectrum obtained for the association (average of the four stations) is so close to Raunkiaer's that it can even be superimposed. The differences do not mean very much as, generally, they

are only 1% except for class A whose difference is 3%. The differences between our spectrum and Kenoyer's and Raunkiaer's normal ones, and also Kenoyer's spectrum for the *Chamaedaphne–Sphagnum* association are as follows:

Classes	A	B	C	D	E
Raunkiaer's normal	− 3%	0	+1%	+1%	+1%
Kenoyer's normal	−19%	+2%	+4%	+5%	+8%
Kenoyer's *Chamaedaphne–Sphagnum* association	− 6%	−8%	+1%	+5%	+8%

With the exceptions of sets 1 and 2 of Lanoraie, all the curves present the "J" shape. Among the 11 graphs here presented, only three follow strictly Raunkiaer's frequency law. The more interesting are the ones obtained for Farnham-total, in which class B is very large and the minimum is in class C; Lanoraie-total, where the record maximum occurred in class C and the minimum in class B; Lanoraie-1 with three maxima, and Lanoraie-2 with the second maximum in class D.

It appears in Table VIII that species like *Kalmia angustifolia* present different indices in the same station. Thus at Lanoraie its frequency varies among the classes B (33%), C (43%), and E (93%), according to the quadrat set. This shows the danger of using a single transect, for if we had taken only transect No. 2, we would have gained a completely wrong idea of the true distribution of the species in the station as measured by the average. If we had disposed the quadrats at random and calculated the index from the assemblage of quadrats, without analyzing them in groups, we would likewise have got an erroneous impression of the distribution which is obviously not at random. In fact, *Kalmia angustifolia* is not equally distributed over all the station's area, as the index shows (55%) based on the total of quadrats, but it is concentrated in part of the station, as can be seen in transect No. 3. Similar testing of other species shows that the majority presents the same non-random distribution as *Kalmia angustifolia*, with the exception of the dominants. This rather forces one to conclude (at least for the *Chamaedaphne* community) that the component species are overdispersed, or better, contagiously dispersed. Several authors (Ashby (4), Clapham (13), Cole (14), Dice (17), Hope-Simpson (26)) studying other communities, including animal communities, have observed this fact.

Because of the given facts, variation of curves and of the species index, we think that the disposition of quadrats at random is the one that gives the best results if the analysis is by several groups of quadrats, in order to show the irregular dispersion of the species.

Constancy and Fidelity

Constancy expresses the occurrence of the species in different examples or individuals of the association (Braun-Blanquet (6), Cain (8)). This concept is confused in the literature with the concept of presence, in spite of its clear

TABLE VIII

FREQUENCY PERCENTAGES AND RELATED DATA FOR THE SPECIES OF THE *Chamaedaphne* COMMUNITY

Station			Lanoraie							
Set No.			1		2		3		Total	
Number of 1 m.² quadrats			30		30		28		88	
Data	L.F.	C%	F%	Class	F%	Class	F%	Class	F%	Class
Andromeda glaucophylla	N	100	10	A	16.66	A	10.7	A	12.4	A
Chamaedaphne calyculata	N	100	100	E	100	E	100	E	100	E
Kalmia angustifolia	N	100	43.33	C	33.33	B	92.8	E	55.3	C
Kalmia polifolia	N	100	60	C	80	D	71.4	D	70.06	D
Ledum groenlandicum	N	100	76.66	D	86.66	E	60.71	D	74.5	D
Vaccinium canadensis	N	100	16.66	A	6.6	A	17.8	A	13.5	A
Vaccinium oxycoccos	Ch	100	33.33	B	70	D	25	B	42.9	C
Carex spp.	H	75	50	C	56.66	C	82.14	E	62.5	D
Carex trisperma	H	75	10	A	2.66	A			12.55	A
Eriophorum spissum	H	75	46.66	C	73.33	D	21.4	B	47.4	C
Nemopanthus mucronata	M	75					14.2	A	4.52	A
Vaccinium angustifolium	N	75	23.33	B	66.66	D	85.7	E	57.6	C
Andropogon sp.	H	50	3.33	A					1.13	A
Aronia melanocarpa	M	50			16.66	A	3.5	A	6.81	A
Betula populifolia	M	50					7.1	A	2.26	A
Carex disperma	H	50			20	A	3.5	A	7.94	A
Larix laricina	M	50								
Rhodora canadensis	N	50	3.33	A	16.66	A	85.7	E	33.9	B
Smilacina trifolia	G	50								
Spiraea latifolia	N	50					3.5	A	1.13	A
Spiraea tomentosa	N	50					3.5	A	1.13	A
Abies balsamea	M	25								
Alnus incana	M	25					3.57	A	1.13	A
Chiogenes hispidula	Ch									
Eriophorum angustifolium	H	25			10	A			4.5	A
Eriophorum virginicum	H	25	3.33	A					1.13	A
Eriophorum sp.	H	25	43.33	C	3.33	A			15.90	A
Gaultheria procumbens	Ch	25								
Glyceria sp.	H	25	3.33	A					1.13	A
Picea mariana	M	25								
Populus tremuloides	M	25	3.33	A					1.13	A
Sarracenia purpurea	H	25	3.33	A	10	A	3.5	A	5.6	A
Senecio sp.	H	25								
Scirpus sp.	G	25								
Viburnum cassinoides	M	25								
Mosses and lichens:										
Aulacomnium palustre		100	3.33	A					1.13	A
Polytrichum strictum		100	100	E	100	E	100	E	100	E
Sphagnum spp.		100	100	E	100	E	100	E	100	E
Cladonia pyxidata		50								
Cladonia cristatella		50	3.33	A					1.13	A
Pleurozium schreberi		50								
Pohlia nutans		50								
Cladonia rangiferina		25								
C. pyxidata var. *chlorophaea*		25	16.66	A	3.33	A	3.5	A	7.9	A
Cladonia sp.		25								
Hypnum crista-castrensis		25								
Polytrichum commune		25								
Points			756.60		872.54		899.22		848.21	
No. of species			23		20		22		31	

definition. It differs from presence in the sense that it is based upon species that occur on a unit area, i.e., in a single quadrat of constant size in each individual stand of the association, while presence is based upon all the area of each stand. In other words, constancy has statistical value, while presence has only descriptive value because different stands have different areas. Constancy is calculated in the same way as frequency except that the latter is based on several plots within one stand and constancy is based on one plot in each stand and the scale used may have 10 or 5 classes. A 5 class scale is used here:

TABLE VIII—*Continued*

FREQUENCY PERCENTAGES AND RELATED DATA FOR THE SPECIES OF THE *Chamaedaphne* COMMUNITY—*Continued*

Farnham								Nominingue		Bellerive		
1		2		3		Total		1		1		
20		30		30		80		30		30		
F%	Class	F%	Class	F%	Class	F%	Class	F%	Class	F%	Class	F points
		16.66	A	10	A	13.33	A	36.66	A	36.66	B	72.39
100	E	100	E	100	E	100	E	100	E	100	E	400.00
75	D	80	D	73.33	D	76.25	D	100	E	96.66	E	328.21
50	C	46.66	C	23.33	B	38.75	B	93.33	E	23.33	B	225.47
55	C	60	C	16.66	A	42.50	C	100	E	100	E	317.00
		20	A	6.66	A	10	A	76.66	D	96.66	E	196.82
		10	A			3.75	A	80	D	23.33	B	149.98
25	B	23.33	B	43.33	C	31.25	B	53.33	C			147.08
				6.66	A	2.50	A			20	A	35.05
		3.33	A	6.66	A	3.75	A	50	C			101.15
10	A	20	A	16.66	A	16.25	A	20	A			40.77
15	A	16.66	A	36.66	B	23.75	B			3.33	A	84.68
								6.66	A			7.79
95	E	46.66	C	93.33	E	76.25	D					83.06
40	B	20	A	36.66	B	31.25	B					33.51
										3.33	A	11.27
								3.33	A	20	A	23.33
		26.66	B	16.66	A	16.25	A					50.15
		6.66	A	10	A	6.25	A	6.66	A			12.91
15	A	3.33	A	40	B	20	A					21.13
5	A			3.33	A	2.50	A					3.63
										6.66	A	6.66
												1.13
3.33	A					1.25	A					1.25
												4.50
												1.13
												15.90
										10	A	10.00
												1.13
								10	A			10.00
												1.13
												5.60
5	A					1.25	A					1.25
										46.66	C	46.66
								3.33	A			3.33
30	B	30	B	13.33	A	23.75	B	33.33	B	60	C	
100	E	93.33	E	96.66	E	96.25	E	93.33	E	60	C	
100	E	100	E	100	E	100	E	100	E	63.33	D	
10	A					2.50	A			3.33	A	
20	A	20	A	3.33	A	13.75	A					
								26.66	B	73.33	D	
								10	A	40	B	
										3.33	A	
5	A					1.25	A					
										6.66	A	
35	B	16.66	A	40	B	30	B					
790		763.27		793.25		781.25		979.92		896.60		
19		22		22		27		27		22		

Class I—present in less than 21% of the stations.
Class II—present in 21% to 40% of the stations.
Class III—present in 41% to 60% of the stations.
Class IV—present in 61% to 80% of the stations.
Class V—present in 81% to 100% of the stations.

Two graphs were constructed (Fig. 9), one based upon the surveys of 1 square meter and the other upon surveys of 100 square meters or smaller areas. It is not possible to produce a graph based on all surveys, owing to the great difference of quadrat area among them. The constancy indices of the species

are recorded in Tables III and VIII. As for frequency, it varies with the number and area of the quadrats used.

Analyzing Table VIII, we find 10 species, including three mosses, that may be considered constants, i.e., that occur in between 81% and 100% of the stations examined. It is well to point out that these results do not correspond exactly to constancy, as they are based upon more than one quadrat for each station. Therefore, this comparison has only relative value and it can not be integrated for the association. The small number of stations (four) does not give an idea of actual constancy of the species through the whole range of the association.

Table III, however, gives data which can be integrated, within limits, for the association. Among the 33 species found, only three (including one moss) can be considered as constants.

On the other hand, the degree of fidelity indicates the more or less rigid limitation of a plant to a certain association (Braun-Blanquet and Pavillard (7), Braun-Blanquet (6), Cain (8)). Its determination is somewhat subjective and depends entirely on the observer's accuracy of knowledge of all the associations present in the region.

According to Braun-Blanquet (6) 5 degrees of fidelity may be distinguished in relation to a certain community.

Fid. 1—Strange species: rare and accidental intruders from another plant community or relicts of a preceding community.

Fid. 2—Indifferent species: without pronounced affinities for any community.

Fid. 3—Preferential species: present in several communities, more or less abundantly, but predominantly or with better vitality in one particular community.

Fid. 4—Selective species: found most frequently in a certain community but also, though rarely, in others.

Fid. 5—Exclusive species: completely or almost completely confined to one community.

We can distinguish, besides the fidelity of a species to the association, its fidelity to the habitat, i.e., its more or less rigid limitation to a certain habitat. In fact, from the ecological point of view, the limitation of a plant to a particular habitat is more important than its limitation to an association. Like fidelity in relation to the community, it may vary according to the geographical region. The scale used is the same, with the only difference that the word habitat will be substituted for community. A good example is arbor-vitae (*Thuja occidentalis*), which is found in the most different habitats, in swamps, along streams, on rocks, on sand, and in old fields, in eastern Canada and northern Michigan (Upper Peninsula), while it is limited to the bogs and valleys with poor drainage, along the rivers, in southern Michigan. The degree of fidelity of a species is thus variable with the region and the ecological range of the species. Plants with a low degree of fidelity in the center of their distribution area may present a high degree on the limits of

TABLE IX

FREQUENCY CLASSES DISTRIBUTION FOR THE *Chamaedaphne* COMMUNITY AT DIFFERENT STATIONS

Class	Farnham								Lanoraie								Bellerive		Nominingue		X		Raun-kiaer	Kenoyer
	1		2		3		*t*		1		2		3		*t*									
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%				
A	7	38.8	12	54.5	10	47.6	14	53.8	12	52.1	10	50	11	50	20	64.5	9	40.9	8	40	199.2	49.8	53	69
B	4	22.2	3	13.6	5	23.8	6	23	2	8.6	1	5	2	9	1	3.2	4	18.1	2	10	54.3	13.6	14	12
C	2	11.1	3	13.6	1	4.7	1	3.8	5	21.7	1	5	—	—	4	12.9	3	13.6	2	10	40.3	10.1	9	6
D	1	5.5	1	4.5	1	4.7	2	7.6	1	4.3	4	20	2	9	3	9.6	2	9.1	2	10	36.3	9.1	8	4
E	4	22.2	3	13.6	4	19	3	11.5	3	13	4	20	7	31.8	3	9.6	4	18.1	6	30	69.2	17.3	16	9
T	18		22		21		26		23		20		22		31		22		20					

their area, or vice versa. *Sarracenia purpurea*, as an example, is selective for the *Chamaedaphne* community in northeastern Canada, and indifferent to the same community in the Bruce Peninsula, Ontario Province. In other words, similar habitats may bear different associations in different floristic areas, and the same species may exhibit different tolerances or association with different communities in different parts of its range. Therefore, fidelity is a local phenomenon (Cain (12)). The combined analysis of fidelity in relation to the community and in relation to the habitat give an exact idea of the eco-sociological behavior of the species.

All the species found in the different surveys (100 square meters and 1 square meter) are listed below with their degree of fidelity in relation to the *Chamaedaphne* association and in relation to the bog habitat.

Species	Fidelity in relation to Chamaedaphnetum	Fidelity in relation to bog habitat
Andromeda glaucophylla	4	5
Chamaedaphne calyculata	4	5
Kalmia polifolia	4	5
Rhododendron canadense	4	5
Vaccinium oxycoccos	4	5
Carex disperma	3	4
Carex trisperma	3	4
Eriophorum spissum	3	5
Eriophorum angustifolium	3	5
Eriophorum virginicum	3	5
Kalmia angustifolia	3	3
Ledum groenlandicum	3	4
Pohlia nutans	3	3
Polytrichum commune	3	3
Polytrichum strictum	3	3
Sarracenia purpurea	3	3
Sphagnum spp.	3	3
Vaccinium angustifolium	3	4
Vaccinium canadense	3	4
Aulacomnium palustre	2	4
Cladonia cristatella	2	2
Cladonia pyxidata	2	2
Cladonia pyxidata var. *chlorophaea*	2	2
Cladonia rangiferina	2	2
Hypnum crista-castrensis	2	2
Pleurozium schreberi	2	2
Smilacina trifolia	2	3
Abies balsamea	1	2
Acer rubrum	1	1
Alnus incana	1	1
Alnus rugosa	1	1
Aronia melanocarpa	1	5
Betula populifolia	1	1
Calla palustris	1	1
Chiogenes hispidula	1	1
Gaultheria procumbens	1	1
Larix laricina	1	3
Myrica gale	1	1
Nemopanthus mucronata	1	5
Picea mariana	1	3
Pinus strobus	1	1
Populus tremuloides	1	1
Rubus chamaemorus	1	1
Salix discolor	1	1
Spiraea latifolia	1	2
Spiraea tomentosa	1	2
Viburnum cassinoides	1	1

Life Forms and Biological Spectrum

The recognition of life forms or growth forms is as old as botanical science. The greatest difficulty encountered by the authors of the different systems is to organize one on a natural basis without creating a large number of classes and subclasses, which make assignment of categories difficult and cause the system to lose much of its usefulness. Raunkiaer's system, because of its simplicity, has become the most popular. In spite of having been improved by several authors, who subdivided the classes and reorganized them, the method is still not ideal for all purposes. Its applicability is easy in temperate climates, but it presents many shortcomings when applied to tropical vegetation.

The biological spectrum, which is the expression in percentage of the number of species in each major life form class, gives the basis for the comparison of the change of the life forms in relation to variations in latitude, altitude, rainfall, etc., and also allows comparison of the floras of different regions.

Raunkiaer (34), having chosen 1000 representative species of the different floras of the earth, found a spectrum, which he called the "normal spectrum." All the comparisons are made in relation to this, which is supposed to represent the spectrum of the earth's flora.

When the spectra are constructed, each species is given the same weight, thus obscuring relative abundance of the species, for rare species have the same value as common and dominant species. Trying to avoid this false representation, Raunkiaer (33) proposed to take the percentages in relation to the total number of species. The points of frequency are obtained by the addition of the frequency indices of the species under consideration. The percentages are obtained by the relationship between the points of frequency for all the species of a life form, and the frequency points of the total number of species found in the sample.

The biological spectrum based on frequency points gives the frequency of life forms in the community. The spectrum constructed this way does not give, however, the value of the cover, or better, the value of the dominance of the different life forms. To avoid this false representation, it was decided to construct a spectrum in relation to the average cover. The procedure is the same as for the construction of the spectrum in relation to frequency, with the only difference that the points are obtained by the addition of the average covers. In both cases the spectrum obtained represents only one side of the problem. When we construct it in relation to frequency we obtain the relative distribution of the life forms in the stand and when we do it in relation to the average cover, we obtain the average value of the cover of life forms. Therefore, to get a real picture of the behavior and value of life forms, it is necessary to make the spectrum not only in relation to frequency, but also in relation to the average cover. For this, it is enough to attribute to each life form a value which is equal to the product of the frequency points by the average cover points. The percentages are calculated on a basis of the total points produced.

The spectra for the different stations and for the total, obtained by the four methods outlined above, are shown in Table X. In this table the item "total flora" corresponds to the total number of species found in the different types of surveys (quadrats of 1 square meter and of 10 square meters).

Analyzing the spectra based upon the species, we see the dominance of the nanophanerophytes. In the spectrum which refers to the group of four stations, the difference between nanophanerophytes and microphanerophytes is of only 3%, and the dominance is observed among the hemicryptophytes. In Lanoraie, the percentage of nanophanerophytes is equal to the percentage of hemicryptophytes, while for the total flora the microphanerophytes are the dominants, the difference between the percentages of them and of the nanophanerophytes being 5%.

In the spectra based upon frequencies, the dominance of the nanophanerophytes and the secondary importance of the hemicryptophytes shows clearly. The spectrum for Farnham shows that the microphanerophytes are important in this station, which corresponds to reality, as the *Chamaedaphne* community is being invaded by *Nemopanthus mucronata* and *Aronia melanocarpa*, pioneers of the following association in the line of normal succession. In the spectrum obtained by grading species only, this fact is obscured by the high number of hemicryptophytes. In the spectrum for Lanoraie, the percentage equality of the nanophanerophytes and hemicryptophytes disappears, the same happening with the dominance of the hemicryptophytes in the spectrum obtained for the group of four stations.

TABLE X

BIOLOGICAL SPECTRA OF THE *Chamaedaphne* COMMUNITY BY DIFFERENT PROCEDURES

	No. vascular species	S	E	MM	M	N	Ch	H	G	HH	Th
Raunkiaer's normal spectrum	1000	2	3	8	18	15	9	26	4	2	13
Spectra based on species											
1. Lanoraie	26	—	—	—	19	38	4	38	—	—	—
2. Farnham	20	—	—	—	15	50	10	20	5	—	—
3. Bellerive	14	—	—	—	14	50	14	14	7	—	—
4. Nomininge	15	—	—	—	27	40	7	20	7	—	—
5. Total for the four stations	35	—	—	—	26	29	18	31	6	—	—
6. Total flora	44	—	—	—	32	27	9	25	5	2	—
Spectra based on frequency											
1. Lanoraie	26	—	—	—	2	64	6	28	—	—	—
2. Farnham	20	—	—	—	24	66	1	8	1	—	—
3. Bellerive	14	—	—	—	4	78	6	4	8	—	—
4. Nomininge	15	—	—	—	5	68	11	15	1	—	—
5. Total for the four stations	35	—	—	—	8	69	7	14	2	—	—
Spectra based on average cover											
1. Lanoraie	26	—	—	—	16	44	4	36	—	—	—
2. Farnham	20	—	—	—	13	57	4	17	9	—	—
3. Bellerive	14	—	—	—	9	64	13	9	5	—	—
4. Nomininge	15	—	—	—	19	55	6	15	5	—	—
5. Total for the four stations	35	—	—	—	14	54	7	21	4	—	—
Spectra based on frequency and average cover											
1. Lanoraie	26	—	—	—	1	72	1	26	—	—	—
2. Farnham	20	—	—	—	7	89	0.5	3	0.5	—	—
3. Bellerive	14	—	—	—	1	96	1	1	1	—	—
4. Nomininge	15	—	—	—	2	90	2	5	1	—	—
5. Total for the four stations	35	—	—	—	3	89	1	7	0	—	—

In the spectra based on average cover, the facts observed are the same as those based upon frequency, with the only difference that the relationships between the percentages for the different life forms are closer to reality.

In the Farnham spectrum based upon frequencies, it appears that the *Chamaedaphne* community is being invaded by microphanerophytes. The spectrum based upon the average cover reveals that these microphanerophytes, in spite of being frequent, have a low average cover, which indicates that the invasion has only begun. The high degree of hemicryptophytes shows that this station has been very much disturbed.

The spectra based upon frequency and average cover combined emphasize the facts of succession mentioned above, but show only the physiognomical character of the association, i.e., the nanophanerophytes' dominance. Although this spectrum gives extreme value, as can be observed on the table, it tends to hide the secondary value of the other life forms.

It is my opinion that, to obtain a true picture of the comportment of life forms in the community, it is necessary to construct all four types of spectra described above.

In the construction of spectra in this study only vascular species have been used and in certain instances different species have been lumped, as in the case of some sterile plants of *Carex*, which could not be identified.

Pichi-Sermolli Maturity Index

The maturity index proposed by Pichi-Sermolli (32) is based upon the theory that the larger the frequency percentage of each species and the smaller the number of sporadic species, the more mature the plant community is. This index is the quotient of the frequency points for all the species found in the community or station being studied by the total number of species.

The author compared two communities: *one* in which there are no dominants and in which some frequent species is accompanied by a great number of sporadic species; *the other* in which there are a few dominants accompanied by a small number of frequent species and a still smaller number of sporadic species. He concludes that the second community is more mature, "not only because the separate species forming it are better distributed, but also because they take advantage of all the possibilities of life offered by the environment" (p. 87).

As the maturity index is based upon the points of frequency, it is subject to the same variations as the frequencies which have already been discussed, i.e., its value differs with the type and number of the quadrats used, as well as with the disposition of these quadrats. For the same reasons, its value varies between 0 and 100 and, the higher the value, the more mature the community.

Table XI gives the data for the maturity indices of the different stations and transects. At the same station the index may vary between 32 and 43. Of the four stations studied, Nomininge may be considered the most mature.

TABLE XI

MATURITY INDICES (PICHI-SERMOLLI) FOR THE DIFFERENT STATIONS AND SETS

Stations	No. 1 m.² quadrats	Points	No. species	Maturity index
Lanoraie:	88	848.21	31	27.36
Lanoraie 1	30	756.60	23	32.89
Lanoraie 2	30	872.54	20	43.62
Lanoraie 3	28	899.22	22	40.87
Farnham:	80	781.25	27	28.93
Farnham 1	20	790.00	19	41.57
Farnham 2	30	763.27	22	34.69
Farnham 3	30	793.25	22	36.05
Nomininguе	30	979.92	20	48.99
Bellerive	30	896.60	22	40.75

Actually, comparing the value of the index with the graph presented in Fig. 9, we can see that the number of species with high frequency is almost equal to the number of the sporadic species.

According to Pichi-Sermolli, this index may be used not only to compare different stands of the same community, but also different associations which replace one another in altitude or latitude. The objective value of this method can only be determined through more extensive study.

Conditions of the Habitat

We can say very little about the physicochemical conditions of the *Chamaedaphne* community, because of the short time taken on these researches. A brief review of bog conditions in general has already been given in a preceding paper (Dansereau and Segadas-Vianna (16)).

The pH of the water found in this community is, generally, low. At Lanoraie bog, the pH of the water in depressions, among *Sphagnum* cushions, was 4.2, while in the water extracted from the sphagna it was 4.8.

The water level remains high. In some stands sphagna are submerged during spring and fall. During the summer, the water is present only in the depressions among cushions. In May, we found an ice layer at about 10 centimeters deep and with a thickness varying between 2 and 5 centimeters. The presence of this layer was noticed at Farnham until May 29, when *Chamaedaphne calyculata* and *Rhodora canadensis* were already in bloom.

Dynamics

The character of the *Chamaedaphne* association is peculiar because it occupies a central position in the normal seres of the vegetation in the bogs (Dansereau and Segadas-Vianna (16)). Upon it converge several seres, some of them induced by human intervention. The development of this association

indicates a phase of consolidation of the bogs, as with it come the sphagna which introduce in the substratum, until now unstable, a great quantity of organic matter, producing a substratum of firmer texture.

The first *Chamaedaphne* individuals are found in pioneer associations, such as the *Carex rostrata* association, *Menyanthes trifoliata* association, *Myrica gale* association, etc., and *Chamaedaphne* persists until late stages of the succession. *Chamaedaphne* stands may be found on lake margins, directly bordering free water, forming a mat, or separated from the open water by several belts of vegetation such as *Carex rostrata* or *Myrica gale*. It is common, however, for stands to be situated in large depressions (Fig. 1) where there is no body of open water. The explanation for the appearance of these large bog areas of several thousand acres is that they are formed through consolidation of smaller bogs (Dansereau and Segadas-Vianna (16)). As the several depressions in an area are filled by peat and as the *Chamaedaphne–Sphagnum* community encroaches on the low areas between the original depressions, there is a paludification of the whole area and a consequent development of a single large bog. The analysis of several profiles (Auer (5), Rigg and Richardson (37)) corroborate this theory because they show the irregular depth of the peat.

In the constitution of this association the first species to become established are *Chamaedaphne calyculata*, *Kalmia angustifolia*, *Kalmia polifolia*, and *Andromeda glaucophylla*. Also, when advancing on free water, their low branches give support to the sphagna, allowing its establishment. In the initial phase the community is open, i.e. presents large, free spaces occupied by water or semiliquid peat, the sphagna limiting themselves to the clumps of *Chamaedaphne*. With the increase of the *Chamaedaphne's* density and consequent augmentation of its support, the sphagna extend not only to the clumps but also into the free areas. With the settlement of species like *Ledum groenlandicum*, the community reaches its intermediate phase. In this phase the upper layer cover is about 80% and the sphagna present a maximum of development, coming to a point where they almost completely submerge the individuals of *Chamaedaphne*, *Ledum*, and *Kalmia angustifolia*.

The final phase is characterized by an augmentation in frequency of *Ledum groenlandicum*, which becomes dominant in the driest places. The disintegration phase begins with the invasion of the community by taller species with higher capacity of competition, such as *Aronia melanocarpa* and *Nemopanthus mucronata*, which gradually eliminate, first the *Kalmia* and then the *Chamaedaphne*. These invaders do not restrict *Ledum groenlandicum*, but on the contrary they increase its cover somewhat, through the whole duration of the subsequent association, dominated by *Nemopanthus*. The disintegration of the community may also be caused by other species, such as *Picea mariana* and *Larix laricina* (Fig. 2), this probably being only a question of chance.

In some stations, apparently because of special edaphic conditions, the *Chamaedaphne* community is long persistent and becomes highly consolidated and resistant to succession. We may say that its cover exceeds 100% because

from the substratum to the tops of the shrubs, all the volume is occupied by *Chamaedaphne* alone. In this case it is a one-layered consociation, and according to Pichi-Sermolli (32) must be considered a mature community *par excellence*.

According to the statements above, the replacement of one association by another is not abrupt but gradual. It is therefore possible to distinguish species which are constructive of the association and others which are destructive. This concept of constructiveness of the species was first proposed by Pavillard (Braun-Blanquet (6)), who recognized seven different types of dynamic behavior for the species of an association.

1—Constructive species.
2—Conserving species.
3—Consolidating species.
4—Conserving and consolidating species.
5—Constructive, conserving, and consolidating species.
6—Neutral species.
7—Destructive species.

As Cain (8) pointed out, the interpretation of these categories is sometimes rather difficult. The list below gives my concept of the dynamic behavior of the species found in this study.

Constructive, conserving, and consolidating species:

Chamaedaphne calyculata
Kalmia angustifolia
Kalmia polifolia
Sphagnum spp.

Conserving and consolidating species:

Aulacomnium palustre
Carex disperma
Carex trisperma
Ledum groenlandicum
Polytrichum commune
Polytrichum strictum
Vaccinium angustifolium
Vaccinium myrtilloides
Vaccinium oxycoccos

Consolidating species:

Hypnum crista-castrensis
Pleurozium schreberi
Pohlia nutans

Conserving species:

Andromeda glaucophylla
Eriophorum angustifolium
Eriophorum spissum
Eriophorum virginicum
Rhodora canadensis
Smilacina trifolia

Neutral species:

Calla palustris
Chiogenes hispidula
Cladonia cristatella
Cladonia pyxidata
Cladonia pyxidata var. *chlorophaea*
Cladonia rangiferina
Gaultheria procumbens
Myrica gale
Rubus chamaemorus
Sarracenia purpurea
Spiraea latifolia
Spiraea tomentosa
Viburnum cassinoides

Destructive species:

Abies balsamea
Acer rubrum
Alnus incana
Alnus rugosa
Aronia melanocarpa
Betula populifolia
Larix laricina
Nemopanthus mucronata
Picea mariana
Pinus strobus
Populus tremuloides
Salix discolor

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CORRECTION

Page 231. In line 16 up, "sélectives" should read "sélectifs".

Notes to Contributors

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